Epidemiological research priorities for public health control of the ongoing global novel coronavirus (2019-nCoV) outbreak

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It is now 6 weeks since Chinese health authorities announced the discovery of a novel coronavirus (2019-nCoV) [1] causing a cluster of pneumonia cases in Wuhan, the major transport hub of central China. The earliest human infections had occurred by early December 2019, and a large wet market in central Wuhan was linked to most, but not all, of the initial cases [2]. While evidence from the initial outbreak investigations seemed to suggest that 2019-nCoV could not easily spread between humans [3], it is now very clear that infections have been spreading from person to person [2]. We recently estimated that more than 75,000 infections may have occurred in Wuhan as at 25 January 2020 [4], and increasing numbers of infections continue to be detected in other cities in mainland China and around the world. A number of important characteristics of 2019-nCoV infection have already been identified, but in order to calibrate public health responses we need improved information on transmission dynamics, severity of the disease, immunity, and the impact of control and mitigation measures that have been applied to date.

Transmission dynamics

Infections with 2019-nCoV can spread from person to person, and in the earliest phase of the outbreak the basic reproductive number was estimated to be around 2.2, assuming a mean serial interval of 7.5 days [2]. The serial interval was not precisely estimated, and a potentially shorter mean serial interval would have corresponded to a slightly lower basic reproductive number. Control measures and changes in population behaviour later in January should have reduced the effective reproductive number. However, it is too early to estimate whether the effective reproductive number has been reduced to below the critical threshold of 1 because cases currently being detected and reported would have mostly been infected in mid- to late-January. Average delays between infection and illness onset have been estimated at around 5–6 days, with

an upper limit of around 11-14 days [2,5], and delays from illness onset to laboratory confirmation added a further 10 days on average [2].

Chains of transmission have now been reported in a number of locations outside of mainland China. Within the coming days or weeks it will become clear whether sustained local transmission has been occurring in other cities outside of Hubei province in China, or in other countries. If sustained transmission does occur in other locations, it would be valuable to determine whether there is variation in transmissibility by location, for example because of different behaviours or control measures, or because of different environmental conditions. To address the latter, virus survival studies can be done in the laboratory to confirm whether there are preferred ranges of temperature or humidity for 2019-nCoV transmission to occur.

In an analysis of the first 425 confirmed cases of infection, 73% of cases with illness onset between 12 and 22 January reported no exposure to either a wet market or another person with symptoms of a respiratory illness [2]. The lack of reported exposure to another ill person could be attributed to lack of awareness or recall bias, but China's health minister publicly warned that pre-symptomatic transmission could be occurring [6]. Determining the extent to which asymptomatic or pre-symptomatic transmission might be occurring is an urgent priority, because it has direct implications for public health and hospital infection control. Data on viral shedding dynamics could help in assessing duration of infectiousness. For severe acute respiratory syndrome-related coronavirus (SARS-CoV), infectivity peaked at around 10 days after illness onset [7], consistent with the peak in viral load at around that time [8]. This allowed control of the SARS epidemic through prompt detection of cases and strict isolation. For influenza virus infections, virus shedding is highest on the day of illness onset and relatively higher from

Research priorities to guide the public health response to 2019-nCoV

Domain	Priorities	Study designs / data sources required
Transmission dynamics	Provide robust estimates of the serial interval and generation time	Detailed exposure and illness onset information from unselected case clusters in line lists, preferably from more than one epicentre
	Estimate effective reproductive number (R_t) in other cities (i.e. ex-Wuhan) in China and elsewhere	Epidemic curves for each city by dates of illness onset, preferably stratified by likely source of infection (zoonotic, environmental point source, local case vs imported index case)
	Clarify the relative importance of pre-symptomatic / asymptomatic transmission	Detailed reports of transmission events and symptomatic status of infectors; viral shedding data; special studies in households and other closed settings
	Determine the role of different age groups in transmission, particularly children	Transmission studies in households and other closed settings; serological studies
	Determine the relative importance of possible modes of transmission	Outbreak investigations, in particular for superspreading events; environmental sampling, air sampling and exhaled breath sampling; special studies in households and other closed settings
	Determine environmental effects on virus survival and transmission	Virus survival studies in situ vivo and in vitro; environmental sampling studies
Severity	Provide robust estimates of the risk of fatality of hospitalised cases, by age or other important groupings	Reports from unselected clinical cohorts of times to death or recovery among resolved cases
	Provide robust estimates of the risk of fatality of symptomatic cases, by age or other important groupings	Estimates of incidence from population-wide surveillance of mild cases
	Identify groups at high risk of severe infection	Case–control studies; cohort studies
Susceptibility	Determine if children are infected, and if so, if they are infectious	Transmission studies in households and other closed settings; serological studies
	Determine if all infections result in neutralising immunity	Convalescent serology from mild as well as severe cases, in all age groups
Control measures	Provide impact estimates of travel restrictions, border screening and quarantine policies on non-local spread	Modelling analyses of local and global spread of infections
	Estimate the effects of social distancing measures and other non-pharmaceutical interventions on transmissibility	Comparative analyses of transmissibility in different locations
	Predict the most effective measures to reduce the peak burden on healthcare providers and other societal functions	Modelling studies incorporating healthcare capacity and processes

shortly before symptom onset until a few days after onset [9]. To date, transmission patterns of 2019-nCoV appear more similar to influenza, with contagiousness occurring around the time of symptom onset, rather than SARS.

Transmission of respiratory viruses generally happens through large respiratory droplets, but some respiratory viruses can spread through fine particle aerosols [10], and indirect transmission via fomites can also play a role. Coronaviruses can also infect the human gastrointestinal tract [11,12], and faecal-oral transmission might also play a role in this instance. The SARS-CoV superspreading event at Amoy Gardens where more than 300 cases were infected was attributed to faecal-oral, then airborne, spread through pressure

differentials between contaminated effluent pipes, bathroom floor drains and flushing toilets [13]. The first large identifiable superspreading event during the present 2019-nCoV outbreak has apparently taken place on the *Diamond Princess* cruise liner quarantined off the coast of Yokohama, Japan, with at least 130 passengers tested positive for 2019-nCoV as at 10 February 2020 [14]. Identifying which modes are important for 2019-nCoV transmission would inform the importance of personal protective measures such as face masks (and specifically which types) and hand hygiene.

Disease severity and immunity
The first human infections were identified through a surveillance system for pneumonia of unknown aetiology, and all of the earliest infections therefore had

pneumonia. It is well established that some infections can be severe, particularly in older adults with underlying medical conditions [15,16], but based on the generally mild clinical presentation of 2019-nCoV cases detected outside China, it appears that there could be many more mild infections than severe infections. Determining the spectrum of clinical manifestations of 2019-nCoV infections is perhaps the most urgent research priority, because it determines the strength of public health response required. If the seriousness of infection is similar to the 1918/19 Spanish influenza, and therefore at the upper end of severity scales in influenza pandemic plans, the same responses would be warranted for 2019-nCoV as for the most severe influenza pandemics. If, however, the seriousness of infection is similar to seasonal influenza, especially during milder seasons, mitigation measures could be tuned accordingly.

Beyond a robust assessment of overall severity, it is also important to determine high risk groups. Infections would likely be more severe in older adults, obese individuals or those with underlying medical conditions, but there have not yet been reports of severity of infections in pregnant women, and very few cases have been reported in children [2].

Those under 18 years are a critical group to study in order to tease out the relative roles of susceptibility vs severity as possible underlying causes for the very rare recorded instances of infection in this age group. Are children protected from infection or do they not fall ill after infection? If they are naturally immune, which is unlikely, we should understand why; otherwise, even if they do not show symptoms, it is important to know if they shed the virus. Obviously, the question about virus shedding of those being infected but asymptomatic leads to the crucial question of infectivity. Answers to these questions are especially pertinent as basis for decisions on school closure as a social distancing intervention, which can be hugely disruptive not only for students but also because of its knock-on effect for child care and parental duties. Very few children have been confirmed 2019-nCoV cases so far but that does not necessarily mean that they are less susceptible or that they could not be latent carriers. Serosurveys in affected locations could inform this, in addition to truly assessing the clinical severity spectrum.

Another question on susceptibility is regarding whether 2019-nCoV infection confers neutralising immunity, usually but not always, indicated by the presence of neutralising antibodies in convalescent sera. Some experts already questioned whether the 2019-nCoV may behave similarly to MERS-CoV in cases exhibiting mild symptoms without eliciting neutralising antibodies [17]. A separate question pertains to the possibility of antibody-dependent enhancement of infection or of disease [18,19]. If either of these were to be relevant, the transmission dynamics could become more complex.

Control and mitigation measures

A wide range of control measures can be considered to contain or mitigate an emerging infection such as 2019-nCoV. Internationally, the past week has seen an increasing number of countries issue travel advisories or outright entry bans on persons from Hubei province or China as a whole, as well as substantial cuts in flights to and from affected areas out of commercial considerations. Evaluation of these mobility restrictions can confirm their potential effectiveness in delaying local epidemics [20], and can also inform when as well as how to lift these restrictions.

If and when local transmission begins in a particular location, a variety of community mitigation measures can be implemented by health authorities to reduce transmission and thus reduce the growth rate of an epidemic, reduce the height of the epidemic peak and the peak demand on healthcare services, as well as reduce the total number of infected persons [21]. A number of social distancing measures have already been implemented in Chinese cities in the past few weeks including school and workplace closures. It should now be an urgent priority to quantify the effects of these measures and specifically whether they can reduce the effective reproductive number below 1, because this will guide the response strategies in other locations. During the 1918/19 influenza pandemic, cities in the United States, which implemented the most aggressive and sustained community measures were the most successful ones in mitigating the impact of that pandemic [22].

Similarly to international travel interventions, local social distancing measures should be assessed for their impact and when they could be safely discontinued, albeit in a coordinated and deliberate manner across China such that recrudescence in the epidemic curve is minimised. Mobile telephony global positioning system (GPS) data and location services data from social media providers such as Baidu and Tencent in China could become the first occasion when these data inform outbreak control in real time.

At the individual level, surgical face masks have often been a particularly visible image from affected cities in China. Face masks are essential components of personal protective equipment in healthcare settings, and should be recommended for ill persons in the community or for those who care for ill persons. However, there is now a shortage of supply of masks in China and elsewhere, and debates are ongoing about their protective value for uninfected persons in the general community.

The Table summarises research gaps to guide the public health response identified.

In conclusion, there are a number of urgent research priorities to inform the public health response to the global spread of 2019-nCoV infections. Establishing

robust estimates of the clinical severity of infections is probably the most pressing, because flattening out the surge in hospital admissions would be essential if there is a danger of hospitals becoming overwhelmed with patients who require inpatient care, not only for those infected with 2019-nCoV but also for urgent acute care of patients with other conditions including those scheduled for procedures and operations. In addressing the research gaps identified here, there is a need for strong collaboration of a competent corps of epidemiological scientists and public health workers who have the flexibility to cope with the surge capacity required, as well as support from laboratories that can deliver on the ever rising demand for diagnostic tests for 2019-nCoV and related sequelae. The readiness survey by Reusken et al. in this issue of *Eurosurveillance* testifies to the rapid response and capabilities of laboratories across Europe should the outbreak originating in Wuhan reach this continent [23].

In the medium term, we look towards the identification of efficacious pharmaceutical agents to prevent and treat what may likely become an endemic infection globally. Beyond the first year, one interesting possibility in the longer term, perhaps borne of wishful hope, is that after the first few epidemic waves, the subsequent endemic re-infections could be of milder severity. Particularly if children are being infected and are developing immunity hereafter, 2019-nCoV could optimistically become the fifth human coronavirus causing the common cold.

Editorial note

This editorial was published as an e-Alert on 11 February 2020.

Conflict of interest

None declared.

Authors' contributions

Wrote first draft: BJC. Critically revised draft and approved final version: BJC and GML.

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Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020

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Timely detection of novel coronavirus (2019-nCoV) infection cases is crucial to interrupt the spread of this virus. We assessed the required expertise and capacity for molecular detection of 2019-nCoV in specialised laboratories in 30 European Union/European Economic Area (EU/EEA) countries. Thirty-eight laboratories in 24 EU/EEA countries had diagnostic tests available by 29 January 2020. A coverage of all EU/EEA countries was expected by mid-February. Availability of primers/probes, positive controls and personnel were main implementation barriers.

In early January 2020, it became evident that a new pathogenic human coronavirus, provisionally named novel coronavirus (2019-nCoV), had emerged in China [1,2]. The virus is causing an outbreak, which started in the metropole Wuhan, but was seeded through travellers across China with ongoing secondary chains of transmission in a wider geographical area. As at 10 February 2020, 40,553 confirmed cases including 910 deaths have been reported worldwide with an increasing number of cases being reported in Europe [3]. So far, instances of secondary spread from international travellers have been limited, but clusters of human-to-human transmission have been reported involving persons with close contact to confirmed cases [4]. A key knowledge gap is the efficiency of community transmission of 2019-nCoV, including the contribution of mild or asymptomatic cases. On 30 January 2020, the Word Health Organization (WHO) declared the outbreak a public health emergency of international concern (PHEIC) because of these uncertainties, the ongoing seeding of the virus internationally, and the need for preparedness across the world in order to track and control the epidemic. WHO highlighted the crucial role of early detection of cases to interrupt virus spread and emphasised that countries need to put in place strong measures to detect and laboratory-confirm cases early [5]. Here, we assessed the required expertise and diagnostic capacity in specialised laboratories in 30 European Union/European Economic Area (EU/EEA) countries.

Survey

A questionnaire was designed to assess the capacity, quality and operational specifics related to 2019-nCoV diagnostics, as well as barriers against their implementation in laboratories that are part of the European Centre for Disease Control and Prevention (ECDC)-associated European expert laboratory network for emerging viral diseases (EVD-LabNet) and/or the European Reference Laboratory Network for Human Influenza (ERLI-Net). The survey was sent on 22 January 2020 to the Operational Contact Points representing 81 laboratories in, among others, 30 EU/EEA countries. The survey subsequently closed on 29 January 2020 (Figure 1). Where indicated, data were validated by individual email exchange with the laboratories to include one entry per laboratory. Entries from laboratories outside the EU/EEA and veterinary laboratories were omitted from analysis for this report. In total, the data provided by 47 laboratories in 30 EU/EEA countries were taken into account in this study.

Time-line with hallmark events of the first two months of the novel coronavirus (2019-nCoV) outbreak, December 2019–January 2020
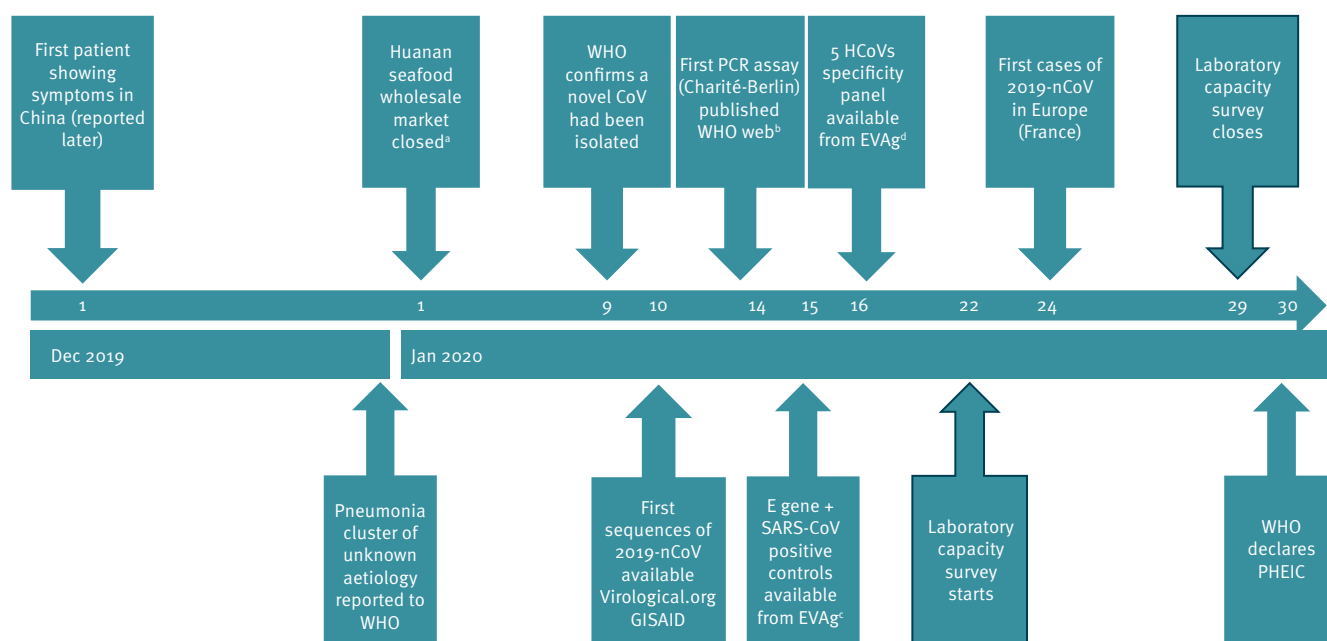
CoV: corona virus; E gene: envelope gene of 2019-nCoV; EVAg: European Virus Archive – GLOBAL; GISAID: Global Initiative on Sharing All Influenza Data; HCoVs: human coronaviruses; 2019-nCoV: novel coronavirus; PHEIC: public health emergency of international concern; SARS-CoV: severe acute respiratory syndrome corona virus; WHO: World Health Organization.

[a] Of the initial 41 people who were hospitalised in Wuhan by 2 January 2020 with pneumonia due to a confirmed 2019-nCoV infection, 27 had an epidemiological link to the Huanan market [18].

[b] https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2

c https://www.european-virus-archive.com/nucleic-acid/wuhan-coronavirus-2019-e-gene-control and https://www.europeanvirus-archive.com/nucleic-acid/sars-cov-frankfurt-1

[d] The five HCoVs included HCoV-NL63, HCoV-OC43, HCoV-229E, MERS-CoV, SARS-CoV. More information is available at: https://www.european-virus-archive.com/nucleic-acid/coronavirus-rna-specificity-panel

Capacity for novel coronavirus molecular diagnostics

At country level, 24 of 30 EU/EEA countries had already implemented molecular tests for 2019-nCoV while the laboratories in the remaining six countries had arranged to ship clinical specimens of suspected cases to a specialised laboratory abroad, while planning to implement assays between 30 January and 17 February 2020. At the laboratory level, 38 of 47 responding laboratories had implemented molecular diagnostics for 2019-nCoV at survey submission, and eight of the nine remaining laboratories planned to have tests implemented by mid-February 2020 (Figure 2). Nineteen laboratories indicated to have capacity to perform whole genome sequencing on 2019-nCoV in clinical samples, while 15 laboratories could perform partial sequencing.

The laboratories were asked to indicate their weekly capacity for molecular testing for 2019-nCoV (Figure 3). Overall, for all 38 laboratories with current capacity this was indicated to be at a minimum of 8,275 tests per week. The eight laboratories in the process of implementing molecular diagnostics would, all combined, add a minimum capacity of 875 tests per week once this process would be complete.

Expertise for coronavirus and other respiratory pathogens

Forty-five laboratories in 28 countries indicated having previous expertise in human coronavirus (HCoV) diagnostics. For two countries the two responding laboratories had no experience. Twenty-five laboratories in 19 countries indicated having experience in molecular diagnostics for all six additional HCoVs (HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E, Middle East respiratory syndrome CoV and severe acute respiratory syndrome CoV) [6]. Forty-four laboratories in 29 countries performed differential testing for other common respiratory pathogens of viral and bacterial origin. Overall, the 47 survey respondents indicated their ability to process a wide range of respiratory sample types, including nasopharyngeal swabs (n = 38),

Figure 2

Status of availability of molecular diagnostics for novel coronavirus (2019-nCoV) in EU/EEA countries as at 29 January 2020 (n = 46 laboratories)[a]
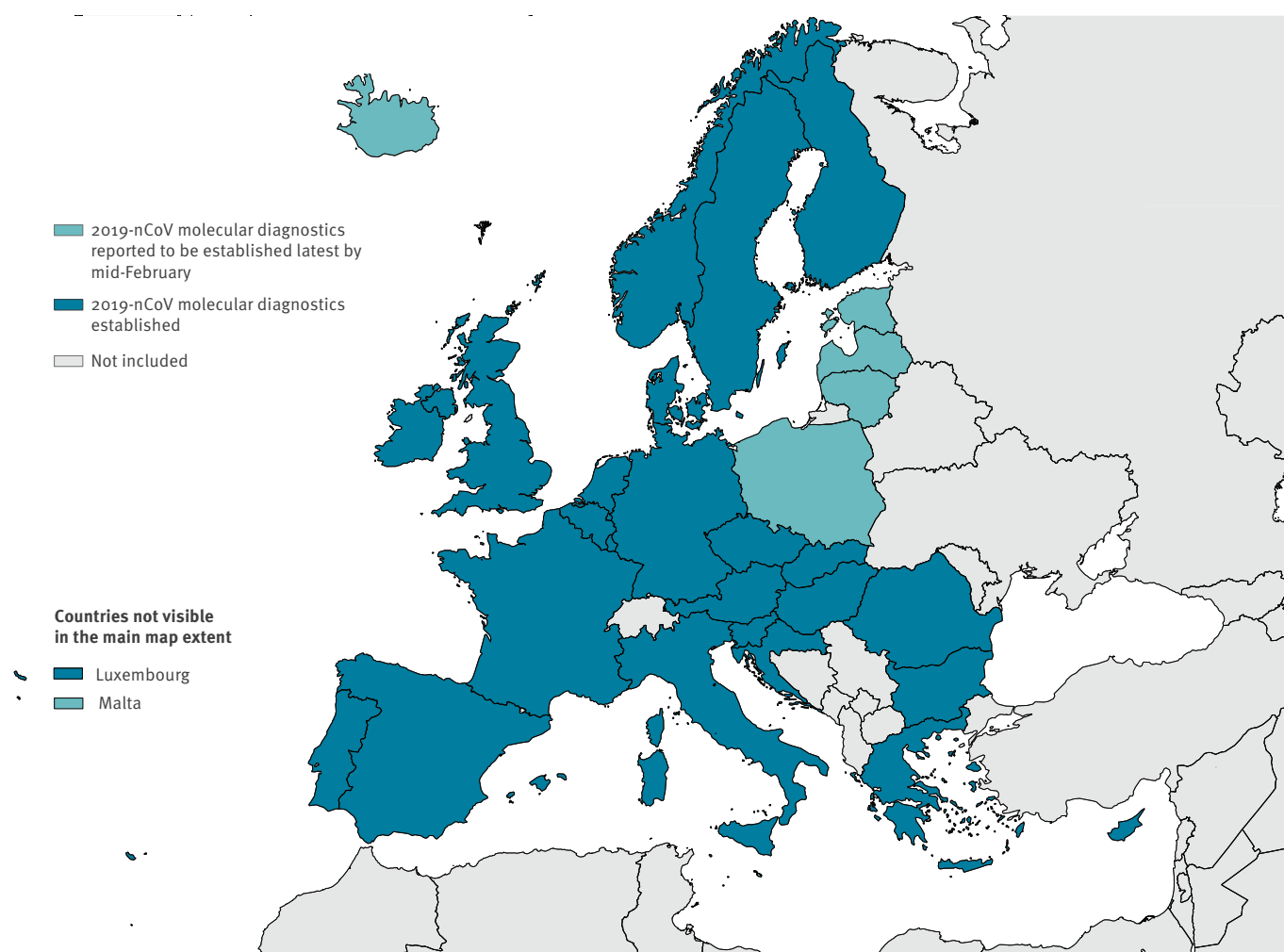
EU/EEA: European Union/European Economic Area; 2019-nCoV: 2019 novel coronavirus.

[a] One laboratory of the 47 included in the current study did not indicate when its molecular diagnostics would be available.

bronchoalveolar lavage (n=36), oropharyngeal swab (n=34), nasopharyngeal aspirate (n=34), sputum (n=34), (endo) tracheal aspirate (n=32) and nasal wash (n=29). In addition, a number of respondents indicated that their laboratories could process biopsy materials (n=28) and whole blood, plasma, serum (n=28) for 2019-nCoV detection.

Implementation of molecular diagnostics for novel coronavirus

Biosafety level

For the biosafety-level (BSL) applied for inactivation of clinical samples suspected of 2019-nCoV, 22 laboratories of the 47 EU/EEA laboratories indicated to do this at BSL2. Twenty-one laboratories indicated to do so at BSL3. Four laboratories indicated an intermediate level BSL2+ (BSL2 with extra precautions such as wearing a filtering face piece (FFP)2 mask). Different approaches

were observed between laboratories within some countries.

Test specifics

As of 14 January 2020, protocols for RT-PCR of 2019-nCoV are being published on the WHO website [7]. At survey closure (29 January 2020), the envelope (E)-gene screening test as published by Corman et al. [6,7], had been implemented by 35 laboratories and the confirmatory RNA-dependent RNA polymerase (RdRp)-gene test and nucleoprotein (N)-gene test by respectively 33 and 21 laboratories. Sixteen laboratories indicated to have additional tests, i.e. in house tests (n=5), pan-CoV tests (n=12) or an assay based on Poon et al. (n=1) [7]. Two laboratories indicated to base 2019-nCoV testing solely on previously published pan-CoV tests [8].

Diagnostic capacity of specialised laboratories with molecular tests available or forthcoming for novel coronavirus (2019-nCoV), EU/EEA, January 2020 (n = 46)[a]

Diagnostic capacity in number of tests per week

■ Molecular diagnostics expected to be available by 17 February 2020

■ Molecular diagnostics implemented at survey closure (29 January 2020)
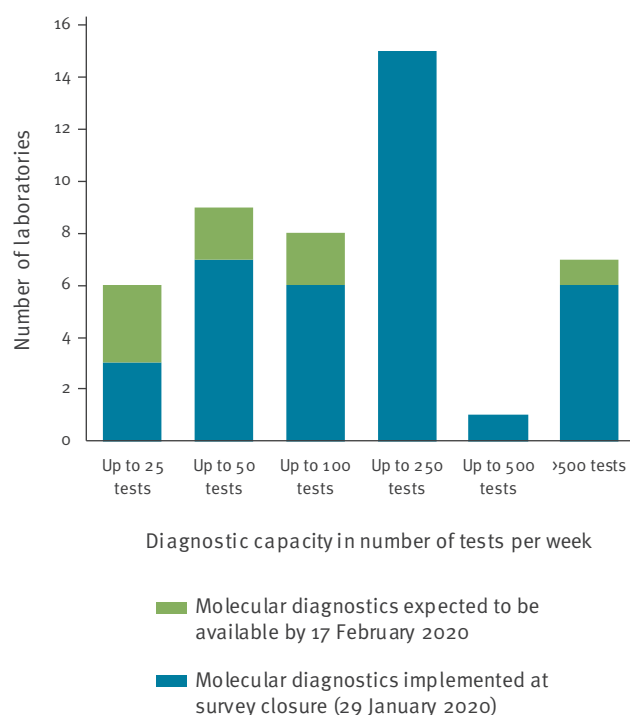
EU/EEA: European Union/European Economic Area.

[a] One laboratory of the 47 included in the current study did not indicate when its molecular diagnostics would be available.

Level of specificity validation

Only 11 laboratories of the 38 laboratories that had implemented testing indicated having validated the specificity of the implemented test against the six additionally known HCoVs and other common respiratory pathogens. For 15 laboratories, specificity validation was still in progress at the time of data submission. Seven laboratories indicated to have only partially validated the implemented test(s) while five laboratories had not (yet) performed any validation. The questionnaire was send out before the first 2019-nCoV cases appeared in Europe (Figure 1) and positive clinical specimens were assumed to be not available to the European laboratories. Therefore, the level of validation for clinical sensitivity was not assessed.

Positive control

Three of 38 laboratories that had implemented diagnostics did the implementation without a positive control. Indicated sources for positive controls were the European Virus Archive (EVAg) (synthetic 2019-nCoV E-gene, SARS-CoV RNA) (n = 23) [9], or own stocks, i.e. SARS-HCoV RNA and/or synthetic RNA (n = 15).

Diagnostic challenges

The top three challenges that were experienced for test implementation were an initial lack of positive control, lack of personnel/time and a lack of primers and/or probes (Figure 4). Nine laboratories in eight countries indicated no obstacles.

Discussion

As at 10 February 2020, 37 confirmed 2019-nCoV cases were reported from eight European countries based on ECDC reporting and testing criteria [3,10]. Multiple modelling studies estimated the risk of 2019-nCoV introduction to Europe as high [11-14]. Pullano et al. indicated the United Kingdom, France and Germany as being at the highest risk, followed by Italy, Spain and the Netherlands [11]. Indeed, all but one country (the Netherlands) have reported cases. The study reported that the occurrence of 2019-nCoV importation from Beijing and Shanghai, both cities with high numbers of travellers to Europe, would likely lead to an even higher and widespread risk for Europe.

This rapid assessment of the readiness of EU/EEA laboratories for molecular detection of 2019-nCoV demonstrated a fast implementation of molecular diagnostics by the European specialised laboratory networks with a good geographical coverage for testing. Among both laboratory networks in this study, protocols were shared rapidly and there was an early availability of positive controls and CoV specificity panels via EVAg. Furthermore, the survey indicated a great willingness of laboratories to provide international diagnostic support [10] and to share sequences to contribute to the monitoring of virus evolution and trace transmission chains.

However, although the first protocols suggesting primer/probe sequences were available fast through the WHO website (Figure 1) and validation panels were made available through the EVAg portal soon after [6,7], the availability of primers, probes and positive controls were indicated as most important initial obstacles for test implementation. In addition, lack of sufficient personnel to implement and validate was a barrier, as had been observed in response to the Zika virus (ZIKV) outbreak in the Americas and the related PHEIC [15]. This suggests that the challenges faced by specialised laboratories when responding to emerging events are of structural nature.

Capacity-wise, the survey indicates that European specialised laboratories are prepared for the current situation, and suggests that a more sensitive case definition than currently in use [10,16] would not create an immediate bottleneck. However, it remains to be seen how realistic the estimates are, particularly in view of the coinciding seasonal epidemic peaks of other respiratory pathogens such as influenza viruses. This will depend on the epidemiological developments in the 2019-nCoV outbreak and on whether the current worldwide control strategy of containment with active case finding [5] will be sustained and the indicated laboratory capacity will suffice. If the outbreak turns into a pandemic, specialised laboratories' efforts would

FIGURE 4

Challenges reported by laboratories in terms of implementing molecular diagnostics for novel coronavirus (2019-nCoV), EU/EEA, January 2020 (n = 47)
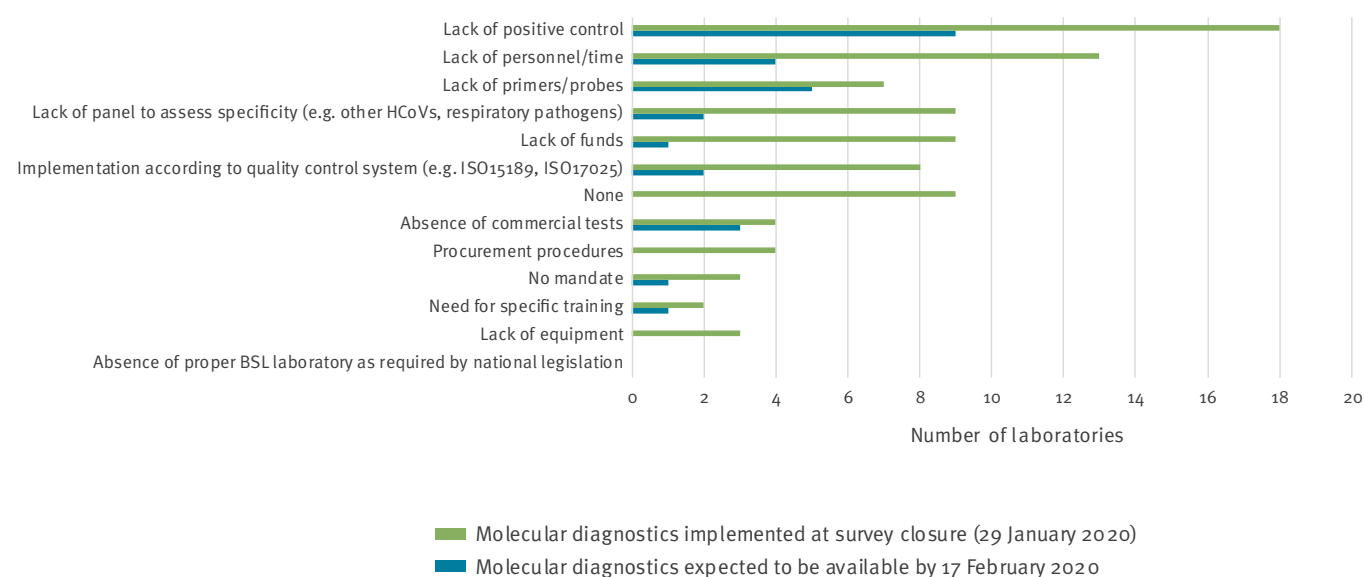
BSL: biosafety-level; EU/EEA: European Union/European Economic Area; HCoVs: human coronaviruses; ISO: International Organization for Standardization.

refocus to reference activities like confirmatory testing, laboratory surveillance including virus characterisation, provision of reference materials and advice, while general testing for 2019-nCoV would shift to first-line hospital laboratories that currently do not have this capacity. This would require a roll-out of tests from the specialised laboratories as was done during the 2009 influenza A(H1N1) pandemic.

The survey showed that proper validation of specificity was lacking in a vast majority of the laboratories that had implemented testing while very few laboratories indicated to have implemented tests without availability of a positive control. The important assessment of the clinical sensitivity of the implemented tests was not possible in this very early phase of laboratory response due to the, at the time, absence of positive clinical materials in Europe. The three laboratories without a positive control will also not have assessed the analytical sensitivity of their tests. The legal possibilities (General Data Protection Regulation; GDPR) for sharing and the willingness to share positive clinical material among the network laboratories now that the first 37 cases have been confirmed in the EU/EEA will determine the speed with which laboratories can address the clinical sensitivity of their implemented tests while the number of cases in the EU/EEA is still limited.

To properly assess the actual capability of the laboratories to detect (sub)clinical 2019-nCoV cases and to provide directions for corrective actions, proficiency testing by external quality assessment (EQA) is essential and urgently needed. The importance of EQA was illustrated in the European ZIKV response where timely implementation was not matched by an overall good capability [17]. Forty of the 47 responding laboratories in this study indicated that they will participate in such an assessment. Currently activities are ongoing to assess the actual capabilities within both laboratory networks by EQA.

In conclusion, while molecular testing for 2019-nCoV was quickly implemented in EU/EEA countries there is still room for improvement especially in the aspect of clinical validation of specificity and sensitivity, as could be expected considering the survey was taken in the very early phase of the laboratory response. Capability testing based on proficiency panels is needed.

Editorial note

This article was published as an e-Alert on 11 February 2020.

Conflict of interest

None declared.

Authors' contributions

CR: survey design, online survey building, data analysis, figures, manuscript writing; EB: survey design, figures, co-writing manuscript; BH, VC, AM, MK, AP, RC, CD: survey design, co-writing manuscript; KL: survey design, data analysis, manuscript writing.

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Prolonged outbreak of New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales (NDM-CRE), Tuscany, Italy, 2018 to 2019

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In Tuscany, Italy, New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales (NDM-CRE) have increased since November 2018. Between November 2018 and October 2019, 1,645 samples were NDM-CRE-positive: 1,270 (77.2%) cases of intestinal carriage, 129 (7.8%) bloodstream infections and 246 (14.9%) infections/colonisations at other sites. *Klebsiella pneumoniae* were prevalent (1,495; 90.9%), with ST147/NDM-1 the dominant clone. Delayed outbreak identification and response resulted in sustained NDM-CRE transmission in the North-West area of Tuscany, but successfully contained spread within the region.

An increase in isolates of NDM-producing carbapenem-resistant Enterobacterales (NDM-CRE) in samples obtained from patients admitted to hospitals in the North-West (NW) area of Tuscany has been registered since the last months of 2018 [1], leading, in March 2019, to the recognition by the Tuscany Regional Department of Health (RDH) of an outbreak situation and to the establishment of a multidisciplinary regional task force (RTF) to coordinate response activities.

Here we describe the spatial and temporal trend of NDM-CRE incidence in Tuscany since the emergence of the outbreak and the public health measures adopted.

Prevention and response activities

Health services in Tuscany, a region of 3.7 million inhabitants in the centre of Italy, are managed by the RDH in three sub-regional areas: NW with a population of 1,200,000 and 3,000 hospital beds, Central with a population of 1,500,000 and 3,000 hospital beds and South-East (SE) with a population of 800,000 and 1,600 hospital beds. Each area comprises one teaching hospital (TH), a number of district hospitals (DH) and smaller hospitals. Following the increase in NDM-CRE cases, the RDH adopted a series of measures (Figure 1) including intensified case detection activities, expanded routine screening of patients admitted to hospitals or specific hospital wards beyond usual practice (from June 2019), definition of standardised

FIGURE 1

Actions to control the outbreak of NDM-CRE cases, by week of implementation, Tuscany, Italy, November 2018–October 2019
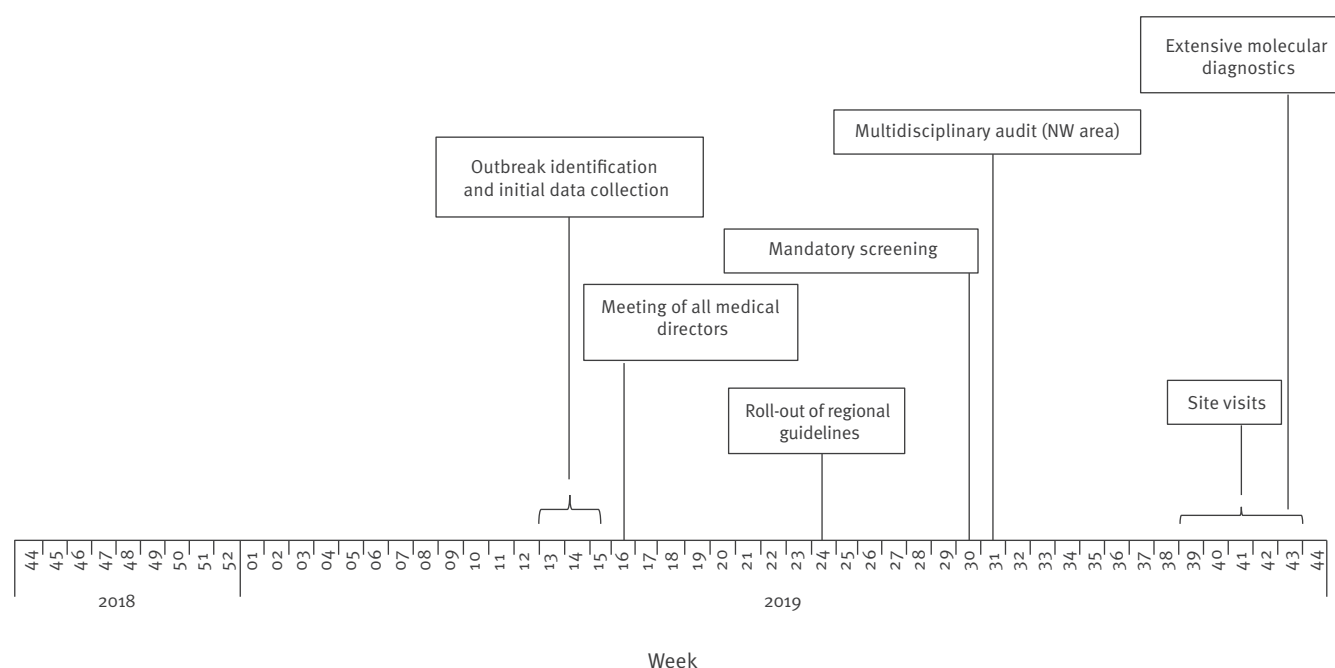
NDM-CRE: New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales; NW: North-West.

procedures for case management, data collection and infection control, and patient safety management aligned with international, national and regional guidelines [2].

Before the introduction of the expanded screening programme of newly admitted patients, the region-wide average number of tests performed per month was 7,500. The number increased to 10,275 in July, 10,974 in August and 19,174 in September, reaching 31,465 in October (Figure 2A). The scale-up of molecular diagnostics use in October 2019 allowed for faster identification of NDM cases. Distribution of the screenings performed by area revealed that half of the samples (51.7%) were collected in the NW area, followed by Central (35.5%) and SE (12.8%), with the higher number in the NW area largely attributable to contact tracing activities in addition to routine screening.

Following detection of NDM-CRE carriage/infection, patients were placed under standard contact precautions. Patient and staff cohorting was implemented where necessary, depending on available infrastructure (e.g. single rooms) and number of affected patients.

Epidemiological investigation

A case was defined as any individual referring to a healthcare facility in Tuscany as an in- or outpatient and for whom the presence of microbiologically confirmed NDM-CRE was detected in any biological sample between 1 November 2018 and 31 October 2019. Cases were classified as: intestinal carriage (isolation from surveillance rectal swabs), bloodstream infections (BSI; isolation from blood samples), colonisation or infection at other sites (isolation from the urinary or respiratory tract or other sites). Individuals with multiple isolations during the same hospitalisation episode were classified according to the most clinically relevant sample.

A dedicated standardised data collection form was developed to gather relevant information on identified cases from November 2018 onwards, including patient characteristics, hospitalisation-related data and microbiological data.

Data were collected by the laboratories and infection control teams of TH and DH and submitted weekly (cases) or monthly (screening samples) to the Regional Health Agency. Data were analysed based on time of isolation, geographical area and hospital or health facility. Cases were stratified by type, bacterial species and, for inpatients, ward or unit (intensive care units (ICU), surgical/medical wards (non-ICU), long-term care units (LTCU)). Rates were calculated as new cases occurring during the study period by total days of hospitalisation per healthcare facility, using estimates for the same period in the previous calendar year. Analyses were performed using STATA/SEv.14.2.

A total of 1,645 cases with NDM-CRE-positive microbiological samples were identified in the period from 1 November 2018 to 31 October 2019. Of these, 1,270 (77.2%) were cases of intestinal carriage, 129 (7.8%)

Number of tests and epidemic curve of NDM-CRE cases by week of detection, Tuscany, Italy, November 2018–October 2019 (n = 1,645)

A. By case type and week of detection; number of rectal swab samples per month

B. BSI cases, by area and week of detection

Legend (A):
- Intestinal carriage (n = 1,270)
- Other (n = 246)
- BSI (n = 129)
- ▲ Rectal swab samples (n = 124,009)

Legend (B):
- North-West (n = 114)
- Central (n = 3)
- South-East (n = 11)
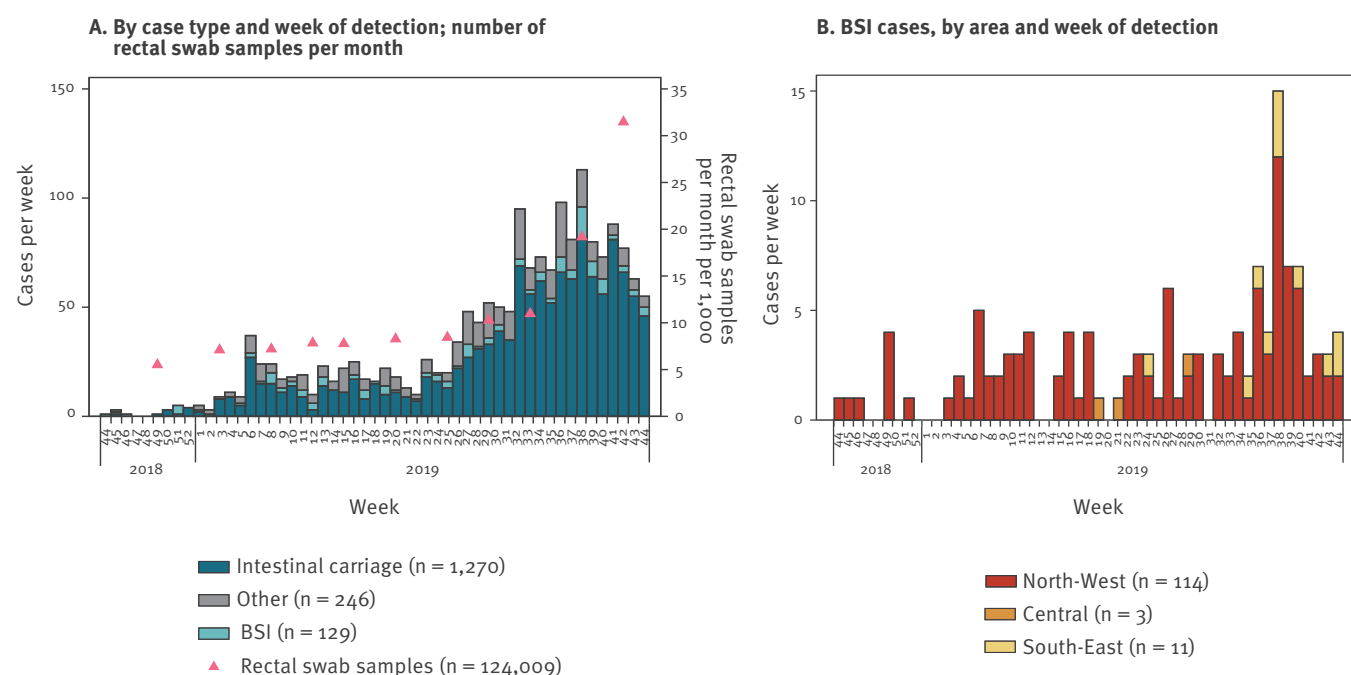
BSI: bloodstream infection; NDM-CRE: New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales.

BSI (one of them from a non-hospitalised patient), and 246 (15.0%) carriage/infection at other sites (urinary tract (180; 10.9%), other sites (66; 4.0%)) (Figure 2). Regarding sex and age distribution, 656 (41.3%) cases were male and 934 (53.7%) female (55 unknown) and the median age was 76 years (range: 0–99 years). There were differences in age distribution (Kolmogorv–Smirnov test) between male (median age: 74 years) and female cases (median age: 80 years).

Most of the cases (1,496; 90.9%) were inpatients (shown in the Table), while a minority (149; 9.1%) were outpatients (e.g. community healthcare services). The majority of all cases (1,391; 84.6%) and of hospitalised cases (1,264; 84.5%) were reported in the NW area, where all TH and DH reported at least one case (data not shown), with the TH and other four DH reporting more than half of the total hospitalised cases in the area (Table). The majority of cases were hospitalised on wards other than ICU at the time of NDM-CRE detection (Table).

According to the available data, a cluster of NDM-CRE cases occurred in November 2018 in the NW area, followed by increasing detection from mid-December 2018. The epidemic curve for the NW area shows an initial peak in mid-February 2019, followed by a further increase over the month of April 2019 (Figure 3A).

The introduction of standardised routine screening of newly admitted patients across Tuscany in June 2019

resulted in a progressive increase in NDM-CRE detection in all TH and DH until September 2019, when a declining trend was noticed (Figures 1 and 3A). The distribution of cases over time was similar across DH in the NW area, with all facilities showing an epidemic curve with multiple peaks (data not shown). Cases in other areas of Tuscany occurred later in 2019 and were fewer (Figure 3B and C). NDM-CRE BSI cases were observed during the entire study period in the NW area, remaining sporadic elsewhere (Figures 2B and 3), as shown in the map (Figure 4).

Microbiological investigation

Microbiological analyses of samples were performed by the TH and DH laboratories following their standard protocols. Identification at the species level was carried out by MALDI-ToF mass spectrometry using either VitekMS (bioMérieux, Marcy-L'Etoile, France) or MALDI Biotyper (Bruker Daltonics, Bremen, Germany) or by biochemical profiling (Vitek2, bioMérieux, Marcy-L'Etoile, France). The presence and nature of carbapenemase determinants was determined by molecular testing of bacterial isolates using either PCR-based platforms (Xpert-CarbaR, Cepheid, Toulouse, France; Allplex, Seegene Inc, Seoul, Korea) or lateral flow immuno-chromatography systems (Resist-5 OOKNV, CorisBio, Gembloux, Belgium). Whole genome sequencing (WGS) was performed on BSI isolates as described previously [3].

NDM-CRE inpatient cases per health facility of detection, Tuscany, Italy, November 2018–October 2019 (n = 1,496)

Area	Hospital type (n)	Total	Confirmed NDM-CRE cases																Rate per 100,000 hospital days[a]
			Per case definition						Per ward										
			Intestinal carriage		BSI		Other		ICU		Other than ICU		LTCU		NA				
			n	%	n	%	n	%	n	%	n	%	n	%	n	%			BSI
North-West	TH (n = 1)	290	231	79.7	45	15.5	14	4.8	52	17.9	219	75.5	4	14	15	5.2			15.46
	DH (n = 4)	727	594	81.7	45	6.2	88	12.1	92	12.7	567	78.0	56	7.7	12	1.7			9.97
	Other[b] (n = 11)	247	178	72.1	24	9.7	45	18.2	17	6.9	108	43.7	108	43.7	14	5.7			12.84
	All facilities	1,264	1,003	79.4	114	9.0	147	11.6	161	12.7	894	70.7	168	13.3	41	3.2			11.84
Central	TH (n = 1)	26	25	96.2	1	3.8	0	0	3	11.5	23	88.5	0	0	0	0.			0.32
	DH (n = 5)	64	58	90.6	1	1.6	5	7.8	12	18.8	47	73.4	0	0	5	7.8			0.19
	Other[b] (n = 8)	27	25	92.6	1	3.7	1	3.7	2	7.4	19	70.4	4	14.8	2	7.4			0.64
	All facilities	117	108	92.3	3	2.6	6	5.1	17	14.5	89	76.1	4	3.4	7	6.0			0.3
South-East	TH (n = 1)	52	37	71.2	4	7.7	11	21.2	11	21.2	41	78.8	0	0	0	0.0			2.35
	DH (n = 2)	12	7	58.3	0	0	5	41.7	2	16.7	9	75.0	1	8.3	0	0.0			0
	Other[b] (n = 13)	51	35	68.6	7	13.7	9	17.6	4	7.8	30	58.8	17	33.3	0	0.0			3.32
	All facilities	115	79	68.7	11	9.6	25	21.7	17	14.8	80	69.6	18	15.7	0	0.0			1.9
Total (whole region)	All facilities	1,496	1,190	79.5	128	8.6	178	11.9	195	13.0	1,063	71.1	190	12.7	48	3.2			5.02

BSI: bloodstream infection; DH: district hospital; ICU: intensive care unit; LTCU: long-term care unit; NA: ward data not available; NDM-CRE: New Delhi metallo-beta-lactamase-producing carbapenem-resistant Enterobacterales; TH: teaching hospital.

[a] Hospitalisation days were calculated based on data for the corresponding months in the previous calendar year.

[b] Other facilities include all hospitals not included in the former categories, e.g. primary hospitals, excluding long-term care facilities.

The large majority of confirmed NDM-CRE were *Klebsiella pneumoniae* (n = 1,495; 90.9%), followed by other Enterobacterales including *Escherichia coli* (n = 69; 4.2%), *Enterobacter hormaechei* (n = 2; 0.1%), *Citrobacter freundii* (n = 1; 0.1%) and unspecified NDM-CRE (n = 77; 4.7%).

Fifty-one unique NDM-positive isolates of *K. pneumoniae* from BSI were characterised by WGS. These isolates were from 12 different hospitals, mostly from the area where the outbreak had emerged. Of these isolates, 49 had been collected during the period from November 2018 to June 2019 and represented 92% of the 53 invasive isolates collected in the same period, while two had been collected earlier (July and September 2018). Most of the 51 isolates belonged to the ST147 lineage and carried the bla_{NDM-1} gene (n = 49; 96%), including the first two isolates collected in summer 2018. Analysis of single nucleotide polymorphisms carried out with core genomes (cgSNP) revealed that all 49 ST147 isolates were closely related to each other (cgSNP range: 1–37; mean: 16; median: 17), suggesting that clonal expansion of a single NDM-1-producing *K. pneumoniae* strain had played a major role at least in the beginning of the outbreak.

Limitations
This study has some limitations. Heterogeneity in screening practice and coverage across the region, imperfect identification of re-admissions and sub-optimal detection of microbiologically confirmed cases before recognition of the outbreak may have affected the accuracy of the present assessment. Furthermore, the reliance on paper-based data collection systems in some facilities and lengthy data collation and validation processes hampered our capacity to extend the present study period beyond week 44 of 2019. While the molecular characterisation data presented in this study are representative of the early phase of the outbreak, further analyses are needed to characterise invasive isolates collected in the second half of 2019.

Public health implications and conclusions
NDM-CRE are able to hydrolyse almost all beta-lactams and are not inhibited by currently available beta-lactamase inhibitors, including the new ones avibactam and vaborbactam [4]. NDM-CRE were first described in 2008 in Sweden and subsequently reported across Europe [1,5], resulting in growing concern for these pathogens to become endemic in the region [6,7]. In Italy, a country where CRE are reported at endemic level [8], NDM-CRE were first detected in 2009 [9,10] and sporadic, often travel-related, cases have been recorded since [5]. This was also the case in Tuscany until November 2018, when a noticeable increase of NDM-CRE isolates was registered [1].

This is a report of a large and persistent outbreak in an Italian region, probably caused by a single-clone

FIGURE 3

Epidemic curve, NDM-CRE cases, by week of detection and area, Tuscany, Italy, November 2018–October 2019 (n = 1,645)

A.North-West area

B.Central area

C.South-East area
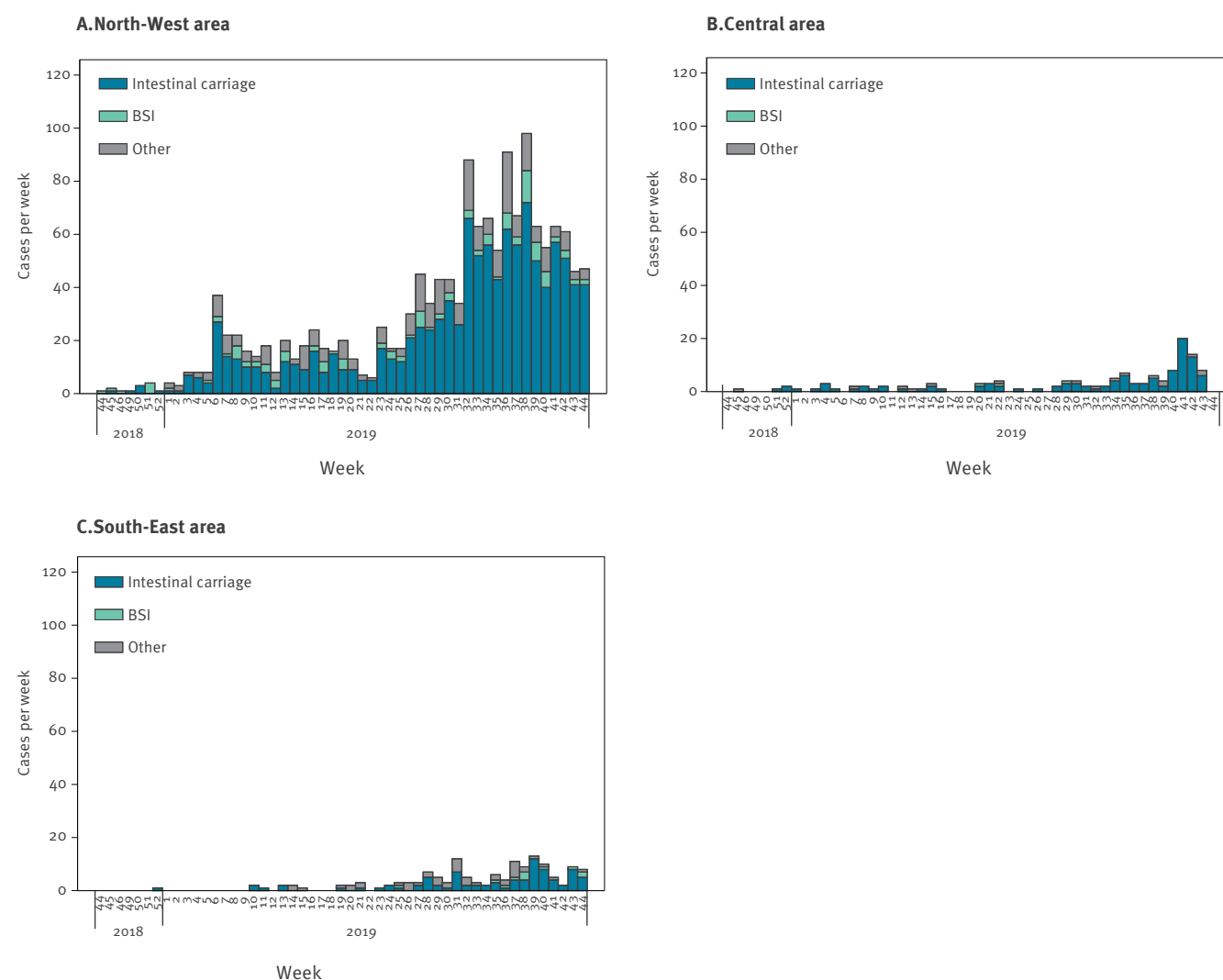
BSI: bloodstream infection.

NDM-CRE, highlighting the risk of rapid emergence and disseminations of uncommon variants of CPE within healthcare facility networks, as recently reported in other European countries [6,7]. Tuscany has a comprehensive and effective microbiological surveillance system, yet the increase in NDM-CRE cases was detected with some delay. This was probably due to the following circumstances: (i) Italy is a setting of high endemicity for CRE and occurrence of CRE isolates was not unexpected, (ii) the routine surveillance system is based on phenotypic resistance profiles to various indicator antibiotics, not including those suggestive of NDM-CRE emergence (e.g. ceftazidime-avibactam), (iii) CRE resistance mechanisms were not routinely searched and (iv) data integration and analysis in the laboratory information systems are not automatised.

Delayed outbreak identification compromised the implementation of a rapidly effective response to contain NDM-CRE spread across healthcare facilities in the

NW area. However, the set-up of a regional RTF and the coordinated roll-out of a comprehensive bundle of interventions was successful in preventing the spread within the Central and SE areas. During the outbreak, infection control protocols were streamlined, combining the contact precautions protocols that were already present with organisational improvements such as cohorting of staff and patients. Most of the efforts were directed towards early case finding and expanding screening protocols in all wards and health facilities. Before the outbreak, routine screening with rectal swabs was performed heterogeneously across Tuscany and largely targeted towards patients admitted to ICU or towards immunocompromised patients, e.g. patients admitted to a haematology ward.

The three sub-regional areas have a nearly closed patient referral system, with patients circulating between the TH and DH/other smaller hospitals located within each area. This referral pathway may

FIGURE 4

Health facilities and numbers of NDM-CRE bloodstream infections, Tuscany, Italy, November 2018–October 2019 (n = 129)
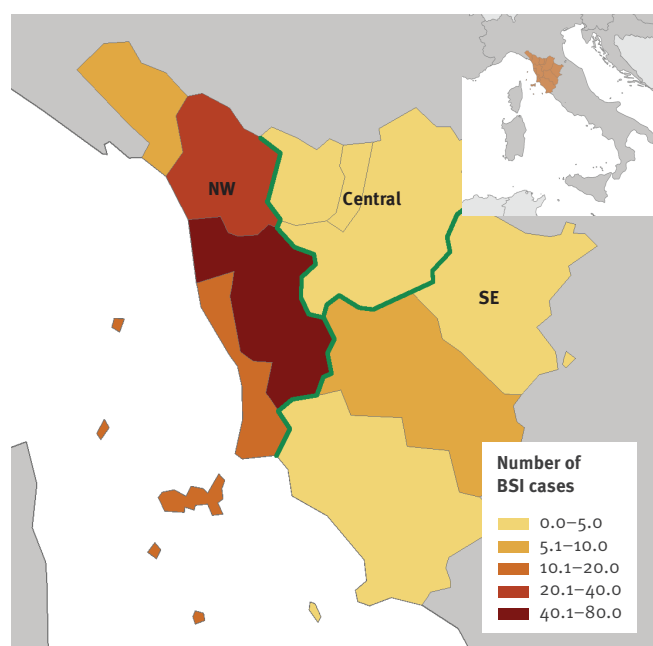
BSI: bloodstream infection; NW: North-West Tuscany; SE: South-East Tuscany.

have enhanced circulation of NDM-CRE through a combination of different mechanisms. While intra-hospital transmission has probably been substantial, inter-hospital and community-to-hospital circulation through patients navigating the health system with multiple admissions across different health facilities are probable reasons for the geographical spread [11,12], at least within the NW area [13]. However, the same self-sufficiency of the Health Services within each area is likely to have contributed to confining the circulation of cases within their borders.

Conclusion

The emergence of NDM-CRE strains in Tuscany, where CRE *K. pneumoniae* circulation within healthcare facilities is already sustained [14], is of great concern as therapeutic options remain very limited. Despite the observed decreasing trend in the number of new cases detected since October 2019, continued monitoring of NDM-CRE transmission is required to assess the risk of further spread within and beyond Tuscany region.

Acknowledgements

Conflict of interest

None declared.

Authors' contributions

LT, SF, AP and LR designed the study, analysed data, drafted and revised the manuscript. GT, GP, PL, FG, MTM, PP, AP and SI contributed to data analysis and manuscript drafting. FP, GG, SS, PV, TB, DT, LT, AB, GL, MP and LR performed the epidemiological investigation and collected data. GMR, FM and MF performed the analysis of microbiological and clinical data and revised the manuscript. The Tuscan Clinical Microbiology Laboratory Network performed the microbiological data collection. All Authors approved the final version of the manuscript.

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First cases of coronavirus disease 2019 (COVID-19) in France: surveillance, investigations and control measures, January 2020

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A novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) causing a cluster of respiratory infections (coronavirus disease 2019, COVID-19) in Wuhan, China, was identified on 7 January 2020. The epidemic quickly disseminated from Wuhan and as at 12 February 2020, 45,179 cases have been confirmed in 25 countries, including 1,116 deaths. Strengthened surveillance was implemented in France on 10 January 2020 in order to identify imported cases early and prevent secondary transmission. Three categories of risk exposure and follow-up procedure were defined for contacts. Three cases of COVID-19 were confirmed on 24 January, the first cases in Europe. Contact tracing was immediately initiated. Five contacts were evaluated as at low risk of exposure and 18 at moderate/high risk. As at 12 February 2020, two cases have been discharged and the third one remains symptomatic with a persistent cough, and no secondary transmission has been identified. Effective collaboration between all parties involved in the surveillance and response to emerging threats is required to detect imported cases early and to implement adequate control measures.

Background

A novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) causing a cluster of respiratory infections (coronavirus disease 2019, COVID-19) in Wuhan, China, was identified on 7 January 2020 [1]. Twenty-seven patients with pneumonia had initially been reported, with an epidemiological link to a live animal market that was closed and disinfected on 1 January [1]. From 20 January, the number of notifications of cases rose dramatically, and as at 12 February 2020, 45,179 cases of SARS-CoV-2 have been confirmed, including 1,116 deaths [2]. Most of the cases (n = 44,665) were reported in 31 provinces and autonomous regions of China and 514 cases were reported in 25 other countries in Asia, Australia, Europe and North America [2]. To date, the primary source of infection remains unknown and could still be active. Human-to-human transmission was observed early after the emergence of this new virus in China and abroad, including family clusters and healthcare settings. The current outbreak dynamics strongly indicate sustained human-to-human transmission.

Strengthened surveillance of COVID-19 cases was implemented in France on 10 January 2020. The

Definition of a contact and follow-up procedure by level of risk of infection, COVID-19, France, January 2020

Level of risk of infection	Contact definition	Follow-up procedure
Negligible risk	Person who had short (< 15 min) contact with a confirmed case in public settings such as in public transportation, restaurants and shops; healthcare personnel who treated a confirmed case while wearing appropriate PPE without any breach identified.	Neither identification nor information of contacts.
Low risk	Person who had a close (within 1 m) but short (< 15 min) contact with a confirmed case, or a distant (> 1 m) but prolonged contact in public settings, or any contact in private settings that does not match with the moderate/high risk of exposure criteria.	Contacts are asked to measure their body temperature twice a day and check for clinical symptoms. In case of occurrence of symptoms like fever, cough or dyspnoea, contacts are asked to wear a surgical mask, isolate themselves and immediately contact the emergency hotline (SAMU-centre 15) indicating that they are contacts of a confirmed COVID-19 case.
Moderate/high risk	Person who had prolonged (> 15 min) direct face-to-face contact within 1 m with a confirmed case, shared the same hospital room, lived in the same household or shared any leisure or professional activity in close proximity with a confirmed case, or travelled together with a COVID-19 case in any kind of conveyance, without appropriate individual protection equipment. Healthcare personnel who treated a confirmed case without wearing appropriate PPE or with an identified breach.	In addition to the above, contacts are asked to stay at home during a 14-day period after their last contact with the confirmed case while symptomatic and to avoid contacts with the other persons living in the same household (or at least wear a surgical mask). The follow-up consists of an active follow-up through daily calls from the regional follow-up team organised by the Regional Health Agency in collaboration with Santé publique France.

COVID-19: coronavirus disease 2019; PPE: personal protective equipment.

objective of the surveillance is to identify imported cases early and to prevent secondary transmission whether in the community or among healthcare workers (HCW). Investigations are carried out among contacts immediately upon illness onset and a follow-up procedure is initiated according to the evaluated level of infection risk.

Here we describe the real-time implementation of this surveillance scheme for the first three imported cases of COVID-19 identified in France, who were confirmed on 24 January 2020 in persons with a recent stay in Wuhan. Two cases were diagnosed in Paris and one in Bordeaux. We present data until 12 February on the follow-up of the cases' contacts initiated immediately upon confirmation of infection.

Methods

French surveillance system
In France, according to the COVID-19 surveillance protocol, physicians suspecting a COVID-19 case have to contact immediately either the emergency hotline (SAMU-Centre 15), if the patient is seeking medical attention from a general practitioner, or a referring infectious diseases specialist at hospital level. Together, they evaluate whether the patient matches the case definition criteria for a possible case (see below). If they do, the case has to be reported immediately through a 24/7 available phone line to the Regional Health Agency (Agence régionale de santé, ARS), which informs without delay the hospital infection control teams involved in the management of the

patient, the French Public Health Agency (Santé publique France, SpFrance) and the Ministry of Health.

A standardised investigation form collecting sociodemographical information, clinical details and history of exposure (history of travel to or residence in Wuhan, China or contact with a confirmed case) is completed for each possible case at regional level, in collaboration between the clinicians, the ARS and SpFrance. Data are entered into the secure web-based application Voozanoo (Epiconcept, Paris).

Possible cases have to be hospitalised, isolated and cared for in one of the 38 French referral hospitals designated by the Ministry of Health, according to the guidelines for the management of patients with Middle East respiratory syndrome (MERS) [3].

For each possible case, respiratory samples from the upper respiratory tract (nasopharyngeal swabs or aspirates) and when possible from the lower respiratory tract (bronchoalveolar lavage fluid, when indicated, or induced sputum) are collected and sent to one of the laboratories accredited to perform SARS-CoV-2-specific real-time RT-PCR. Until 27 January, only the National Reference Centre for respiratory viruses (Institut Pasteur, Paris) was able to test for the presence of the SARS-CoV-2.

Case definition
From 17 to 29 January 2020, a possible case was defined either as a patient with a severe acute lower respiratory infection requiring admission to hospital and with a history of travel to or residence in Wuhan,

Timeline of travel, onset of illness and close contacts of confirmed cases of COVID-19, France, January 2020 (n = 3)
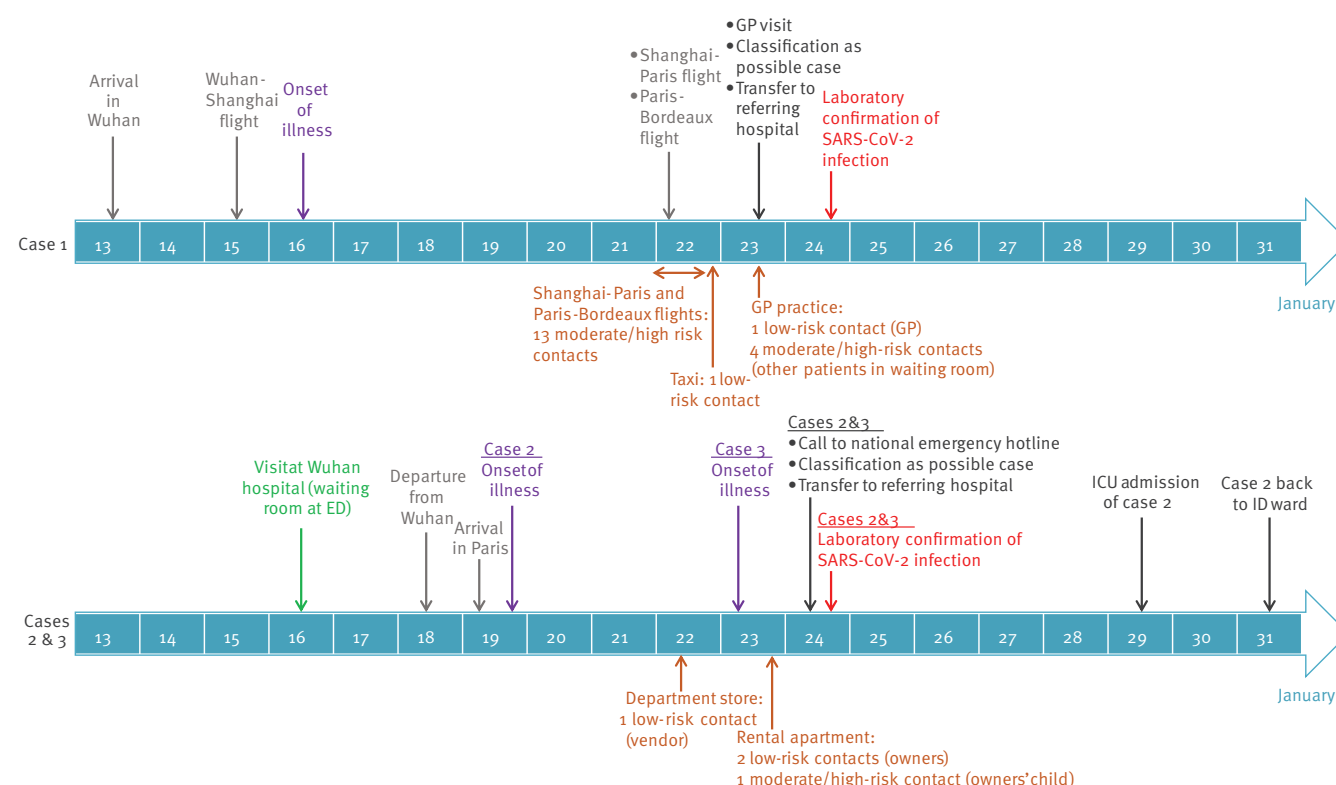
ED: emergency department; GP: general practitioner; ICU: intensive care unit; ID: infectious diseases.

China in the 14 days before symptom onset, or a patient with an acute respiratory illness whatever the severity and with a history of at-risk exposure, mainly to a confirmed case. A confirmed case was defined as a possible case with a positive SARS-CoV-2 RT-PCR on respiratory samples, performed by an accredited laboratory. Testing relied on the real-time RT-PCR procedure developed by the Charité [4] as well as on the use of real-time RT-PCR specific for the RdRp gene (four targets) designed at Institut Pasteur (RdRp-IP).

The case definition was first set up on 10 January and adapted over time. The detailed case definition used for the cases presented here as well as the most up-to-date case definition are available in the Supplement.

Contact and co-exposure tracing

Co-exposed persons are defined as people who shared the same risks of exposure as a possible or confirmed case of COVID-19. Contact and co-exposure identification is done for all identified possible cases. Contacts are traced from the date of onset of clinical symptoms in a case. If the diagnosis of SARS-CoV-2 infection is confirmed in the index case, active surveillance of contacts/co-exposed persons is initiated immediately.

Three levels of risk of infection are defined for contacts/co-exposed persons of a possible/confirmed COVID-19

case (Table). Co-exposed persons of a confirmed case are followed-up according to the same procedure as a moderate-/high-risk contact. The follow-up procedure for the contacts/co-exposed persons differs according to the evaluation of the level of risk of infection (Table). During the initial implementation phase of the procedure, owing to the limited number of contacts involved, it was decided to also implement an active follow-up for low risk contacts.

Patients are interviewed by the clinicians, with the help of a translator if needed, who recover relevant information on their contacts since onset of clinical symptoms and the nature and intensity of exposure. The involved regional health agencies work closely with the regional entities of Santé publique France (cellules régionales) in order to implement contact tracing and follow-up. Santé publique France coordinates the surveillance at national level in liaison with the national Health Authorities.

Ethical statement

The investigations were carried out in accordance with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78–17 on 06/01/1978 and Décret 2019–536 on 29/05/2019). Informed consent to disclosure of information relevant to this publication

was obtained from the three patients confirmed with 2019-nCoV infection.

Results

Detected confirmed cases

Between 10 January and 24 January (period until confirmation of the first cases in France), nine possible cases were identified in France; among them, three cases were confirmed with COVID-19.

Case 1 was a 48-year-old male patient living in France. He was travelling for professional reasons in China in various cities including Wuhan when he experienced his first symptoms (fever, headaches and cough) on 16 January. He flew back to Bordeaux, France on 22 January via Shanghai, Qingdao and Paris Charles de Gaulle airports. He reported wearing a mask during the flights. He sought medical attention from a general practitioner on 23 January, where he was suspected of COVID-19, and was subsequently transferred to the regional referring hospital in Bordeaux, isolated and sampled for laboratory confirmation of SARS-CoV-2 infection. Infection was confirmed on 24 January by the National Reference Centre (Figure). Case 1 tested positive only for the E gene target when using the Charité procedure [4] and was positive for all four RdRp-IP targets with threshold cycles (Ct) in good agreement with those obtained for the E gene target.

The patient arrived in Wuhan on 13 January, did not report any visit to markets, exposure to live animals or contact with sick persons during his stay. No detailed information is available about the circumstances of exposure, apart from a visit to family members and friends on 15 January.

Case 2 was a 31-year-old Chinese male tourist who had left Wuhan on 18 January and arrived in Paris on 19 January. He developed fever, chills, fatigue, conjunctivitis and cough on 19 January. Case 3 was a 30-year-old Chinese female tourist who travelled with Case 2. She developed fever, chills, fatigue and cough on 23 January. On 24 January, they were advised by the Chinese embassy to seek medical attention at the national hotline (SAMU-centre 15) and were immediately transferred to a regional referring hospital, isolated and sampled for laboratory confirmation of COVID-19. Infection with SARS-CoV-2 was confirmed on 24 January for both of them by the National Reference Centre (Figure). Cases 2 and 3 were positive by RT-PCR for all targets of the Charité procedure [4] (RdRp Pan Sarbeco and 2019-nCov probes; E; N) as well for the four RdRp-IP targets with Ct values in good agreement with those obtained for the E gene target.

The condition of the male patient deteriorated on 29 January and he was admitted to the intensive care unit (ICU) the same day. He stayed 72 h in the ICU for non-invasive oxygen therapy and was transferred back to infectious diseases ward on 31 January.

Neither of the two cases reported any visit to markets, exposure to live animals or contact with sick persons during the 14 days before symptom onset. Both visited a hospital in Wuhan on 16 January for an unrelated medical condition in Case 3 (Figure).

As at 12 February, Case 1 was afebrile and symptomatic with a persistent cough. Cases 2 and 3 were not symptomatic any more and were discharged from hospital on 12 February.

As soon as the infection with SARS-CoV-2 was confirmed for the three cases on 24 January, this information was immediately released through a press conference held by the French Minister of Health and the Chief Medical Officer. Daily public communication on the state of the investigations around the cases was subsequently implemented by the Ministry of Health. Daily updates were also published on the SpF website.

The three cases were notified to the European Commission via the Early Warning and Response System (EWRS) on 26 January, and to the European Center for Disease Prevention and Control (ECDC) via the European Surveillance System (TESSy) on 28 January.

Contact and co-exposition tracing

No co-exposed person was identified for Case 1. Two contacts were evaluated at low risk of infection, the taxi driver who drove the case from the airport to his home (30-min drive) and the general practitioner who took care of the patient before wearing appropriate personal protection equipment (3-min non-close contact). Seventeen contacts were evaluated at moderate/high risk of infection. Four of them shared the same waiting room in the general practitioner's office while Case 1 was coughing, seated ca 1–1.5 m away from the case during 5–30 min. The other 13 contacts were the persons sitting in the two seats around Case 1 in the Shanghai–Paris and Paris–Bordeaux flights (Figure). They were considered at moderate risk of exposure despite the fact that Case 1 reported wearing a mask during the whole flight; this was based on the length of one of the flights (>6 h) and the fact that it was unclear whether or not Case 1 removed his mask during short periods (e.g. meals) and kept the same mask during the whole flights. None of the contacts of the Shanghai–Paris flight were French nationals and their contact tracing was referred to their home countries' health authorities. All other identified contacts were evaluated at negligible risk of infection because the contacts were short and/or distant in public settings and did not imply face-to-face conversations or because appropriate personal protective equipment (PPE) was worn by the healthcare personnel who took care of the patient, including those involved in the transfer from the general practitioner to the referring hospital.

Cases 2 and 3 stayed together and shared the same activities during their stay in Paris, and therefore

shared the same contacts from 23 January (date of illness onset for Case 3). Three contacts were evaluated at low risk of infection: the two owners of the apartment rented by the couple and a department store employee with whom Case 2 reported a distant (>1 m) contact during around 20 min on 22 January. The apartment owner's child who visited Cases 2 and 3 and was hugged by them was evaluated at moderate/high risk of infection (Figure). All other identified contacts were evaluated at negligible risk of infection, as contacts were short and distant in public settings such as department stores and did not imply face-to-face conversations or because appropriate PPE was worn by the healthcare personnel who took care of the patients.

Follow-up of the identified contacts was initiated according to the COVID-19 procedure (Table). As at 2 February, two contacts have been classified as possible cases since the implementation of the follow-up: A person sitting two seats away from Case 1 during the Paris–Bordeaux flight, and therefore identified as a moderate/high risk contact, developed respiratory symptoms on 27 January and was classified as a possible case on 31 January and was subsequently excluded following negative RT-PCR results. Infection with SARS-CoV-2 was excluded on the same day. A radiology assistant who took care of both Cases 2 and 3 developed respiratory symptoms on 30 January and was classified as a possible case on 2 February. This person had been classified as at negligible risk of exposure, because she wore appropriate PPE during the whole procedure. Infection with SARS-CoV-2 was excluded on 2 February. Follow-up of the contacts ended on 6 February. No identified contact of the three cases has been confirmed with COVID-19.

Discussion

Specific COVID-19 surveillance has been in place in France since 10 January 2020, 3 days after the identification of the SARS-CoV-2 in China. The first three imported cases of COVID-19 in France, the first ones in Europe, were diagnosed 14 days later, on 24 January. Rapid and effective collaboration between the clinicians (general practitioners attending the cases, emergency hotline clinicians (SAMU-centre 15) and infectious diseases specialists), the National Reference Centre and the regional and national health authorities has played a crucial role in the system's capacity to quickly detect, isolate and investigate those cases in order to implement adequate control measures. The surveillance system as well as the control measures were adapted from those implemented during past emerging infections that occurred after 2003 (severe acute respiratory syndrome (SARS), MERS, influenza A(H1N1)pdm09, Ebolavirus disease), and all involved parties were already familiar with the system, which probably favoured its responsiveness.

The case definition of a possible case in use on 24 January was slightly adapted from the one provided by the World Health Organization (WHO), based on

an epidemiological link to Wuhan, China and a severe lower acute respiratory disease. It is noteworthy that the first nine possible cases identified in France, including the three confirmed cases described here, displayed mild respiratory symptoms with no sign of severity at the time of diagnosis. Increasing evidence suggests that mild clinical symptoms could be more frequent in cases of COVID-19 than with SARS-CoV and MERS-CoV [5]. Therefore, the case definition in effect on 24 January lacked sensitivity. This was counter-balanced by a tendency from the infectious diseases specialists in charge of classification of suspected cases to privilege the exposure to Wuhan over the clinical presentation in their decision. However, we cannot exclude that some COVID-19 cases remained undetected in France because of the lack of sensitivity of our case definition. The clinical criteria were expanded on 4 February to include any lower acute respiratory disease and the epidemiological criterion was extended to the whole of China. At that time, the French laboratory capacities were reinforced from one to five laboratories able to perform the diagnostics for COVID-19. Further extension to all 38 referring hospital laboratories is expected by early to mid-February 2020. Santé publique France will deploy in early February the outbreak investigation tool developed by the WHO (Go. Data [6]) in order to facilitate case data management and contact tracing at the national and local level in France.

Contact and co-exposure identification of the three confirmed cases had been initiated as soon as they were classified as possible cases, which facilitated investigations upon confirmation of COVID-19. Confirmation of the diagnosis was made in the evening of 24 January and the investigation to retrieve as exhaustively as possible contacts and co-exposed individuals and evaluate their level of risk of transmission was started immediately overnight. Complete transparency of the investigations was ensured through daily press conferences held by the French health authorities.

Although the follow-up procedure for the contacts/co-exposed persons used in France slightly differ from the ECDC and WHO guidelines [7,8], which were not available at the time of this investigation, it relies on the same general principles. Contact tracing of the passengers seated near Case 1 during the two flights Shanghai–Paris and Paris–Bordeaux was adapted from the ECDC guidelines for infectious diseases transmitted on aircraft [9]. Even though Case 1 was wearing a face mask during those flights, we could not exclude breaches and subsequent risk of transmission to the persons sitting in the two seats around him.

Because of the current uncertainties about the capacity of SARS-CoV-2 to easily spread from human to human, the decision to consider a contact as close if the case–contact distance was between 1 m and 2 m was made on a case-by-case basis, depending on the type and length of interaction. Through the extensive

interviews made with the cases and their high compliance to cooperate to the investigation, we believe that the contacts most at risk have been satisfactorily identified. All of them could be rapidly contacted and informed about measures to be taken, which they all agreed to. However, some contacts were either impossible to trace back (e.g. co-travellers on public transportation) or evaluated as at negligible risk of exposure because of short and/or distant contacts (e.g. restaurant, contacts with cashiers while running errands, visiting museums), although accidental events carrying the risk of transmission on such occasions, such as an episode of cough of sneezing, cannot be ruled out.

Moreover, the contact tracing was limited to the period after onset of illness. However, should the transmission of SARS-CoV-2 occur during the asymptomatic phase, we cannot exclude that secondary transmission events initiated from the three confirmed cases remained undetected during the investigations.

Case 3 developed symptoms 4 days after her husband and 5 days after the couple had left Wuhan. The incubation period of SARS-CoV-2 is currently estimated at around 3–7 days [5,10,11]. Therefore, she may have acquired the infection from her husband, although this cannot be proved.

The active surveillance of close contacts of confirmed COVID-19 cases and the implementation of control measures, including home quarantine for those evaluated at moderate/high risk of exposure, decrease the risk of human-to-human transmission originating from imported cases and subsequently delay propagation of the virus in the general population. This allows our healthcare system to prepare for any further spread of the epidemic. Besides, the epidemiological and clinical data collected about the confirmed cases and their contacts will increase our knowledge of COVID-19.

The rapid and collaborative management of the first imported COVID-19 cases in France highlights the fact that the French healthcare system is adequately prepared to respond to such emerging diseases threats. However, this surveillance system is extremely time-consuming and requires considerable manpower. The data available on 12 February strongly suggest that human-to-human transmission of SARS-CoV-2 is frequent, with the reproduction number estimated at 2 to 3 [5,10-14]. Twenty-five countries have already reported imported cases from China, and several of them have described autochthonous transmission events [15]. In the case of further spread of SARS-CoV-2 worldwide, it would soon become impossible to detect all imported cases and trace their contacts. Especially the occurrence of large clusters in the same region would strongly impact on the local health authorities' capacities. The surveillance objectives would then need to evolve from containing the epidemic to mitigating its medical and societal impact.

As at 12 February, the contacts of the three first confirmed cases of COVID-19 in France have been followed up for the whole 14 days follow-up time after the cases isolation. No secondary transmission event has been detected so far despite active follow-up. Given the first estimations of the SARS-CoV-2 incubation period, the probability of secondary cases originating from those three cases is negligible.

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Conflict of interest

None declared.

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Cyber security threats in the microbial genomics era: implications for public health

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Next generation sequencing (NGS) is becoming the new gold standard in public health microbiology. Like any disruptive technology, its growing popularity inevitably attracts cyber security actors, for whom the health sector is attractive because it combines mission-critical infrastructure and high-value data with cybersecurity vulnerabilities. In this Perspective, we explore cyber security aspects of microbial NGS. We discuss the motivations and objectives for such attack, its feasibility and implications, and highlight policy considerations aimed at threat mitigation. Particular focus is placed on the attack vectors, where the entire process of NGS, from sample to result, could be vulnerable, and a risk assessment based on probability and impact for representative attack vectors is presented. Cyber attacks on microbial NGS could result in loss of confidentiality (leakage of personal or institutional data), integrity (misdetection of pathogens) and availability (denial of sequencing services). NGS platforms are also at risk of being used as propagation vectors, compromising an entire system or network. Owing to the rapid evolution of microbial NGS and its applications, and in light of the dynamics of the cyber security domain, frequent risk assessments should be carried out in order to identify new threats and underpin constantly updated public health policies.

Introduction

Next generation sequencing (NGS) is an emerging technology in the field of public health microbiology [1]. Whole genome sequencing (WGS) of pathogens has recently gained acceptance as a new gold standard in microbiology for different pathogens and scenarios; it allows the unprecedented characterisation of pathogens with respect to taxonomy, antimicrobial resistance, virulence attributes and genotyping [2]. Among many other advantages, it is expected to reduce the time from diagnosis to clinical treatment, improve surveillance and outbreak investigation and facilitate data sharing in public health [3]. The adoption of WGS is rapidly increasing thanks to a dramatic reduction in the cost of DNA sequencing [4]. The continuous development in the field of metagenomics suggests that NGS could soon be harnessed on a routine basis for culture-independent microbiology, which is expected to further improve surveillance and management of infectious diseases [5].

As with any disruptive technology, growing popularity of a technology will inevitably attract the interest of malicious actors who will try to abuse it, at individual or state level. Painfully bright examples of this recurring pattern involved major disruptions in Internet services worldwide [6] or malicious software specifically designed to steal cryptocurrency wallets in the wake of Bitcoin's rise [7]. The collective experience in the field of cybersecurity so far suggests that for a new technology not to become an immediate hazard, security should be integrated as early as possible and periodic security audits should be carried out throughout its whole lifecycle [8]. The costs of sequencing continue to drop, allowing efforts to introduce sequencing globally, even into low resource settings. Moreover, small footprint benchtop sequencers and, even more importantly, portable sequencers are being developed [9]. These trends indicate that in the near future, increasing proportions of microbial sequence data will be generated outside of the traditional laboratory setting, such as in the field during investigation, at the bedside and even in consumer homes and other unorthodox locations (e.g. in outer space [10]).

In this Perspective, we explore cyber security aspects of microbial NGS. We discuss the motivations and objectives for a possible attack, its feasibility and implications, and highlight policy considerations aimed at mitigating this growing threat.

Cyber threat assessment in public health microbiology
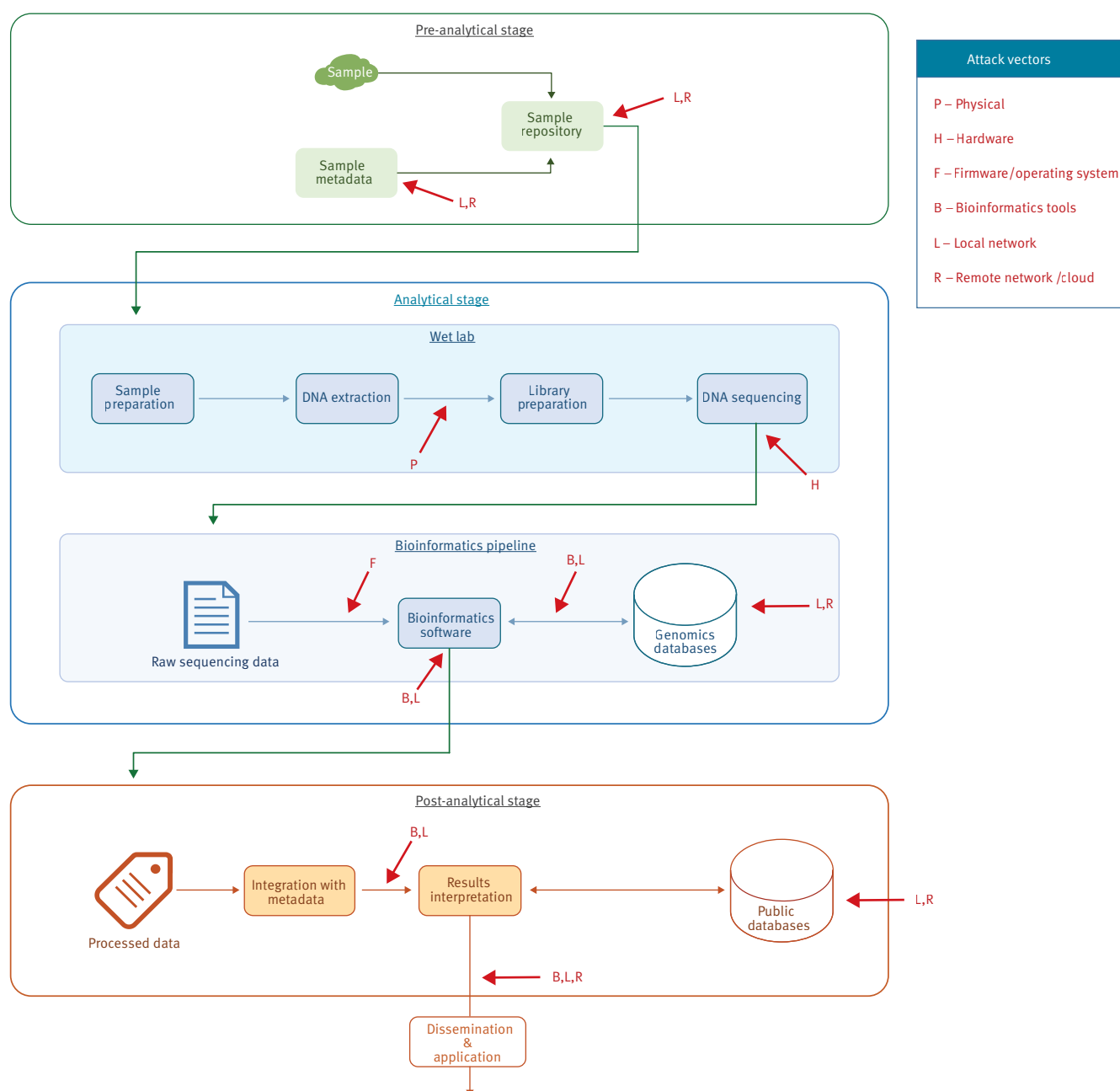
Schematic representation of a sample-to-result microbiological workflow in the public health microbiology setting. The workflow is divided into the pre-analytical, analytical and post-analytical phases of the diagnostic cycle. Red arrows represent vulnerabilities in the different phases of the process to different cyber attack vectors.

Medicine and cyber security

In recent years, a sharp rise in cyber attacks on smart medical equipment had been observed [11] as part of the more general trend of increased cyber attacks on Internet-connected devices, including smart home devices such as locks, cameras, lights and speakers. Computerised medical equipment is an attractive target for malicious cyber activity, as it is among a rapidly shrinking group of industries which combine mission-critical infrastructure and high-value data (e.g.

personal health records), with relatively weak cybersecurity standards [12]. In the context of medical devices, cyber threats could be targeting a specific facility or organisation, such as the recent incident that involved hospitals in the United Kingdom [13], or involve a supply chain attack targeting less secure elements in an organisational supply network [14]. An adversary might carry out a supply chain attack by first compromising a network or device-providing service [15]. Cyber security must therefore be a core part of a medical product's lifecycle and, in particular, integrated into the product's

Cyber threat analysis relevant to next generation sequencing in public health

Attack vector	Methods	Target NGS stage	Required access
Physical	Malicious biological material	Sample preparation	Physical
Hardware	Hardware implant	Sequencing	Interdiction/manufacturing
Firmware/operating system	Firmware replacement	Sequencing Bioinformatics	Physical Interdiction/manufacturing Compromised PC
Software	Targeted infection Supply chain	Bioinformatics	Compromised PC Local Network Remote
Local network	Targeted infection Supply chain Data breach	Bioinformatics	Compromised PC Remote
Cloud infrastructure	Data breach	Bioinformatics	Remote

NGS: next generation sequencing; PC: personal computer.

design from its inception and not as an afterthought. Traditionally, the responsibility for the security of medical devices lies with the device manufacturer, while the responsibility for sensitive information is in the hands of medical institutions.

The rapid growth of machine learning applications and data analytics in medicine are also of great concern with respect to cyber security, especially in the face of adversarial learning – an advanced offensive technique designed to fool models based on machine learning that is applicable to medical information technology systems [16]. Recent studies in the field of adversarial learning have demonstrated successful attacks on medical devices such as imaging technology [17]. In an era of digital transformation of healthcare, cyber threats are unavoidable and effective cyber security requires a major investment in infrastructure, personnel and governance [12].

While cyber attacks on microbial NGS have not been reported to date, a practical attack has been performed compromising a computer as a part of an NGS pipeline via a specially synthesised DNA sequence [18], which suggests that this avenue deserves more attention and that microbial NGS has unique cyber security aspects that go beyond generic IT aspects. Of note, the malicious sequence was processed by an NGS device (an Illumina NextSeq), but the sequencer itself was not used as a propagation vector nor was it compromised. Rather, it was the NGS device's proper functionality that permitted the attack in the first place.

Attack vectors

A schematic representation of the public health microbiological workflow appears in the Figure , involving sample preparation, sequencing and bioinformatics analysis stages [19]. The bioinformatics analysis usually involves an output or end result, which is interpreted and communicated to relevant stakeholders [20]. Table 1 describes the different attack vectors and methods applicable to a generic NGS process. An adversary can attack at multiple stages of the NGS pipeline, with different attacks requiring different access levels (e.g. physical, local network, remote network). This analysis highlights the need for policymakers to employ cyber security best practices throughout the NGS diagnostic cycle, starting from the acquisition of biological material and ending in cloud-based bioinformatic applications. The analysis shown in Table 1 is generic – different NGS platforms use a variety of technologies and architectures, making some of the threats relevant only to a subset of currently available platforms. All stages of the NGS process, from sample preparation to post-sequencing bioinformatics analysis, could be vulnerable to cyber attacks.

Table 2 presents a risk assessment for representative attack vectors at the different stages of the NGS process. The probability and impact of each attack are ranked on a scale of 1 to 5, each based on the expert opinion of the authors. High-probability scores were awarded to threats that require minimal access to carry out, have higher technological feasibility and for which stronger incentives exist among adversaries. High-impact scores were awarded to threats resulting in overall system compromise and particularly to those which made it possible to use the host PC as a cyber attack propagation vector and to threats with a wider national or international impact. Following the Common Vulnerability Scoring System (CVSS) 3.1 methodology [21], an overall score for each vector was obtained by multiplying its probability and impact scores. The different threats were then categorised into three groups

TABLE 2
Probability and impact assessment of representative cyber attack vectors

Attack vector	Method	Possible impact	Impact scale	Required access	Mitigating factors	Impact	Probability	Score
Biological processing	Synthesis of malicious biomatter that would compromise device or sequencing software	From false results to full system compromise	Devices sequencing malicious biomatter	Access to biological samples to be sequenced by device	Chain of custody as biomatter is handled; software protections in sequencer	5	1	5
Signal processing	Flash malicious bitstream/ hardware replacement	Misdetection of bases, false results	Single device	Physical access	Binding and tamper-proofing sequencer, signing and authenticating field upgrades	4	3	12
	Flash malicious firmware on hardware subsystem	Misdetection of bases, false results	Single device	Access to a PC connected to the sequencer		4	3	12
Proprietary hardware components	Feed sequencing software with false results	False-negative or false-positive result	Single device	Possibly accomplishable remotely	Authenticate device-PC communications	5	4	20
	Attack sequencing PC	Malicious code running on PC	Single device; possible propagation/ escalation vector	Possibly accomplishable remotely	Standard practices for protecting PCs	5	5	25
Sequencing/ bio-informatics software	Flash malicious firmware on subsystem	Misdetection of bases, false results	All devices in contact with malicious PC; possible propagation/ escalation vector	Access to a PC connected to the sequencer; possibly accomplishable remotely	Authenticate device-PC communications	5	3	15
	Display false sequencing results	False-negative or false-positive on detection of disease			Standard practices for protecting PCs	5	3	15
	Infect PC with targeted malware to interfere with sequencing software operations	False-negative or false-positive detection of disease; Ability to infect other devices and PCs	All devices and PCs on the same network as the malicious PC; network propagation/ escalation vector		Restrict and regulate interface between PC and sequencer	5	2	10
Sequencer and related equipment (e.g. PC)	Propagate malware using sequencer as an infection vector	PCs in proximity of sequencer infected with malware	All PCs in contact with infected sequencer	Access to a PC connected to the sequencer; possibly accomplishable remotely	Restrict and regulate interface between PC and sequencer	4	4	16
	Leak of sensitive personal data	Leak of sensitive personal data	Owner of sample/data		Standard practices for protecting PCs	2	5	10
	Report false data to the sequencer cloud	False data accumulated at scale, false global information	Commercial/public data repositories		Authenticate PC-cloud communications	2	1	2
Cloud services	Deliver malicious sequencer firmware or sequencing software at worldwide scale	Malicious software deployed at scale	All user base of a cloud, network propagation/ escalation vector allows arbitrarily large infection scale	Remote	Standard practices for protecting cloud services	5	1	5

PC: personal computer.

Impact scale: 1 – minimal public health impact; 2 – local or limited consequences; 3 – moderate or severe local consequences; 4 – national consequences; 5 – severe national or international consequences.

Probability scale: 1 – minimal feasibility; 2 – limited feasibility and/or incentive; 3 – moderate feasibility and/or incentive; 4 – high feasibility and/or incentive; 5 – high feasibility, imminent.

according to the overall score, with scores ranging from 1 to 5 being considered minor threats, 6 to 15 representing moderately dangerous threats and scores of 16 to 25 representing major threats. A total of 12 threats have been included in the analysis, containing six main attack vectors comprising of several adversarial methodologies. Of these, three were deemed major, six moderate and another three minor threats. Attacks pertaining to peripheral or proprietary hardware present the most dangerous combination of required access, attack impact and probability and required resources, followed by attacks on sequencing software. Table 2 also includes a selection of factors that can mitigate the highlighted threats. Some factors, such as protecting PCs and cloud servers, are generic IT best practices, while some are specific to the NGS domain and its use of connected sequencing hardware.

Attack objectives

The International Organization for Standardization (ISO) standards body defines in ISO/IEC 27000 a set of principles for the operation of a secure system: confidentiality, integrity and availability [8]. In the specific domain of NGS devices, several high-level motivations for an adversary can be considered according to these principles.

The **confidentiality** principle stipulates that a system must ensure that information is not made available or disclosed to unauthorised entities. In the context of NGS, attacks on confidentiality include data leakage of medical records, and especially of genetic information, which are considered to be highly personal and sensitive and thus of very high value. Data leakage may occur through the action of an outside attacker, but it may also occur through internal misuse (the 'angry administrator' scenario). Liabilities with respect to data safety and security are even more pronounced in light of the recent introduction of the general data protection regulations (GDPR). In the least harmful scenario, targeted advertising could take advantage of a person's medical situation, maybe even without their awareness, to make profit. In a more concerning scenario, personal medical records of high-profile targets could be used to extort, blackmail or even physically harm them.

Beyond the individual level, leakage of raw sequence data or results of sequencing procedures, could result in an embarrassment to public health institutions, especially if information has not yet been properly analysed, or if information is presented out of context without relevant metadata and expert interpretation.

The **integrity** principle stipulates that a system must protect the accuracy and completeness of information. In the context of NGS, attacks on integrity include misdetection attacks, in which the device could appear to be functioning, while in effect, it provides false results to the user. Attacking a core sequencing facility intended for public health purposes, could lead to erroneous diagnosis and, as a consequence, mistreatment of patients or inconclusive investigation. Such a scenario would carry grave consequences both to individual patients and to medical and public health facilities. Significant economical and reputational damages should be taken into account in such situation.

Maintaining the integrity of devices is particularly important when they are used in an incident response scenario. As misdetection could result in a false alarm, e.g. an Ebola outbreak could be 'detected' while no actual virus was present, leading in an extreme case scenario to a public health response, disruption of routine and critical services, disruption of normal business, public panic and disorder and mobilisation of government resources to contain a non-existent outbreak. In an arguably worse-case scenario, misdetection may involve a false-negative result, meaning the sequencing procedure would report the sample as harmless, while it actually contained a significant biological threat.

The **availability** principle stipulates that a system should be accessible and usable when an authorised entity demands access. Denial of service is a form of attack in which a device, process, or facility is rendered unavailable. In our specific context, sequencing devices could be arranged to fail under certain conditions. At the very least, such an incident imposes an economic penalty on a victim organisation. Furthermore, an unexpected failure of devices during a biological incident can significantly delay or even deny appropriate public health response.

At the IT infrastructure scale, attackers may attempt to compromise a weakly secured device as a stepping stone for infiltrating a different network or system. In this scenario, the real objective of the attack will not be to attack the NGS device itself, but rather to achieve system or network compromise. In such an attack, the NGS device is used as an infection and propagation vector for advancing the attacker's position to target a machine, facility or network associated with the device. This attack is common to all connected devices and is not unique to NGS devices. NGS devices, however, are mainly used in government and medical facilities, arguably two of the highest-risk sectors regarding cyber activity, making this threat important to consider. Moreover, the increasing popularity of mobile sequencers further augments this vulnerability.

It is also important to note that while attacks carried out on a single device would have a moderate impact at best, if deployed at scale, attacks may create a sustained incident on a national or even global level.

Attack scenarios

Here we propose a number of possible attack scenarios and discuss the resources and skills required to carry them out.

Biological substance attack

As demonstrated by Ney et al. [18], synthesising a malicious DNA sample to carry out an attack on a sequencing PC is technically feasible. That said, extensive knowledge of both computer science and microbiology is required to carry out such an attack, along with carrying out extensive security evaluation of the sequencing software to find a potential vulnerability. Furthermore, the malicious DNA sample should be tailored for the specific sequencing device on which the sample would end up, a non-trivial piece of foreknowledge. Finally, the question of how the sample would end up being synthesised by the device in the first place leads to scenarios involving field-deployed human agents or collaborators on the victim side. Those assumptions lead us to rate this threat as having a low probability of taking place. Nevertheless, the probability of such attack could increase in the future, depending on technological advancements.

Malicious hardware/firmware implant

In this scenario, attackers manage to be in a position where they can communicate with the device locally, through serial or networked connections, or can physically disassemble it. Recent reports testify to the ability and motivation of state actors to place themselves in such positions [15,22]. It is not uncommon for workers of various sectors to use their company's PCs for various personal activities, thus increasing the chance of infection by malware from the Internet: an NGS device compromised at time of manufacturing or by interdiction could serve as an infection vector for computing systems in a medical or government facility, but a PC infected ahead of time and controlled by the attacking party could be used as a remote implanting station for the NGS devices in its vicinity. In a typical public health laboratory setting, a small number of NGS devices will communicate with numerous PCs as part of sequencing and bioinformatics analysis stages, and so both directions are efficient propagation vectors. Most devices are typically protected from infection by IT security safeguards such as malware protection and secure coding practices. Medical devices, however, are known to be more sensitive to malware and low-quality code than other connected devices, owing to the lengthy compliance process that makes in-the-field upgrades very difficult [12]. Finally, embedded device firmware has been shown to suffer often from poor security mechanisms and thus is more susceptible to various forms of attacks than traditional computer systems [23]. The various factors described above lead us to believe that this attack scenario is highly probable.

Next generation sequencing software compromise

Software is known to contain vulnerabilities caused by imperfect code, misconfiguration etc., and NGS-related software, used to operate sequencing and laboratory equipment or carry out the bioinformatics analyses, is no exception. Software vulnerabilities are exploited to gain unauthorised access to computer systems or networks, leak data, crash or otherwise disrupt various services. In the NGS context, vulnerable sequencing software could be made to malfunction, report false results or serve as an initial foothold on a medical or government facility's network. If the application runs with high privileges or makes use of other high-privilege software components (e.g. a device driver), this scenario could lead to full system takeover. A remotely exploitable vulnerability could lead to a remote attacker controlling sequencing PCs across the world. At scale, this would mean any device which installed the sequencing application would serve as an entry point to its system and the network it attaches to.

A different attack vector using the NGS software would be a supply chain attack similar to an incident reported in 2017 [24], in which the online software repository used to distribute a popular application was compromised, and the hosted application was replaced by a malicious version of itself. All instances of the application downloaded from the repository would infect their host PCs with malware. A similar incident can occur with the repository hosting software powering a benchtop or a portable sequencer. According to a recent audit of popular sequencing software packages performed by Ney et al. [18], those applications generally suffer from bad security hygiene practices and thus finding an exploit in one of them is highly feasible.

Policy implications

The field of microbial genomics is vulnerable to cyber threats and therefore, there is a need to develop and implement a suitable policy to mitigate such threats. The main components of such policy may include the following:

- Cyber security aspects should be taken into account when local, national or international surveillance systems based on genomics are designed and implemented.

- NGS devices are not simple, passive devices – they contain active computing and networking capabilities and should thus be appropriately considered by IT policy. Good general IT and information security organisational practice is important to protect against many of the risks described herein.

- An ongoing dialogue between scientists and practitioners and IT and security personnel is needed in order to identify cyber threats related to newly developed and introduced technology.

- Skills and capacity building in cyber security should be considered by public health institutions and should be introduced to formal education programmes as well as on-the-job training.

- The possibility of a cyber attack should be taken into account during outbreak detection and

investigation and explored further by specialists if deemed relevant.

- Manufacturers of laboratory equipment, particularly DNA sequencing technology, should consider cyber security threats during platform development, manufacturing and marketing.

- Developers of commercial or open source bioinformatics software should consider cyber security threats during software development and testing.

- Surveillance tools, capable of detecting or predicting cyber attacks involving DNA sequencing should be developed and implemented in surveillance networks.

- The impact and probability of the various attack vectors should be evaluated more broadly while consulting a range of experts from related fields in different countries, in order to fine-tune and validate risk assessments.

Given the rapid evolution of DNA sequencing technology and its applications for microbial genomics and in light of the dynamics of the cyber security domain, frequent risk assessments should be carried out in order to identify new threats and update public health policy aimed at mitigating those risks.

Conflict of interest

None declared.

Authors' contributions

Inception – JMG, YO. Literature review and analysis – IF, YO, YM, JMG. Drafting of paper – IF, JMG. Critical review of draft – LR, YM, YO.

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